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From the Department of Medicine (Professor W J Kalpainen) and the Department of Obstetrics and Gynaecology (Professor Pentti A. Järvinen) University of Oulu Oulu Finland

ACUTE INTERMITTENT PORPHYRIA AND PREGNANCY

BY

JUHANI SALOKANNEL AND KURT RHEN

Porphyria usually refers to a hereditary disorder of porphyrin metabolism, characterized by increased secretion of porphyrins in the urine Large amounts of porphyrin metabolites are also found in the urine in some diseases and intoxications e.g in severe liver diseases, anaemia cardiac infarction and heavy-metal intoxications (*Watson* 1963 *Kiss* and *Szemere* 1965 *Schmid* 1967)

At least fifteen forms of porphyria are recognized today On the basis of clinical and biochemical differences, the porphyrias may be divided into two major groups erythropoietic porphyria, in which the abnormal production of uroporphyrins occurs in normoblasts of the bone marrow and hepatic porphyria, in which excessive amounts of porphobilinogen and uroporphyrins are synthesized in the liver The most common is acute intermittent porphyria (AIP) which belongs to hepatic group (*Watson* 1963 *Harber* 1965) *Waldenström* (1957) reported an incidence of 1 5 cases of AIP per 100,000 inhabitants in Sweden. The incidence in Finland has been presumed to be the same as in Sweden (*Koskelo* 1962)

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The onset of acute intermittent porphyria is usually heralded by abdominal colic, although neurological symptoms, such as weakened tendon reflexes, and pareses of the peripheral and autonomous cerebral nerves, are common. In some patients the psychic symptoms, such as hallucinations and psychoses, predominat

AIP is inherited, according to the Mendelian laws of inheritance dominantly autosomally (*Waldenström* 1937) The incidence in women is approximately 1 5 times that in men (*Watson 1954 Waldenström 1957)* The most common age of onset is between 20 and 30 years (*Waldenström* 1957) in other words the fertile age of women. For this reason occasionally a women with porphyria is pregnant and the possible influence of pregnancy and parturition on the course of porphyria must be assessed

The Department of Medicine and Department of Obstetrics and Gynaecology of University of Oulu have treated several members of one family for acute intermittent porphyria. The purpose is here to describe the effect of pregnancy and child birth on their disease and to advance an opinion on the basis of the experience thus gained and on the findings reported in the literature concerning the role of pregnancy in AIP

Case reports

The family immigrated from East Karelia The father was born May 26 1907 and is still living He has had pain in the back and lower limbs for over 15 years and been treated for a sciatic syndrome Sometimes after urinating in snow he noticed that the urine was red. His macroscopically clear urine was examined for porphyrins for the first time in 19.6 in connection with the present study none were demonstrable and sediment was normal

The mother of the family was born March 18 1906 and exhibited no symptoms nor signs suggestive of porphyria.

No porphyria could, therefore be demonstrated objectively in either of the parents although the father s aches and the occasional red colour of the urine justify a suspicion that he had latent AIP

The family had 13 children. Three had died before they were 3 years of age As far as is known, the causes of their deaths were infectious diseases One daughter died at the age of 31 of acute intermittent porphyria The three sons living were healthy and had shown no symptoms suggestive of porphyria Of the six daughters living, AIP has been diagnosed to date in five

The eldest child in the family a daughter born April 24 1929 had normal deliveries in 1951 and 1953 AIP was diagnosed in 1956 at the beginning of her third pregnancy with abdominal pain as the first symptom. Subsequently the pregnancy continued uneventfully and delivery as normal About a month *post partum* the abdominal pain recurred and the patient urine turned dark in colour The pain subsided in a few days but the patient became psychotic and was treated at the Central Mental Hospital of Oulu for

nearly three weeks. A year after this delivery the patient again became pregnant and from the beginning of the pregnancy complained of pain on both sides of the body. In the third month of gestation the pregnancy was terminated because of increasing symptoms of AIP and the patient was sterilized. She recovered rapidly and is still living. She has not needed treatment in hospital for porphyria.

The next daughter was found to have AIP at the age of 30 in 1951. The first symptoms were abdominal pain and vomiting, and the urine turned dark in colour. She had two miscarriages in 1956 and 1959 and two deliveries in 1958 and 1960. In connection with the latter delivery she was given pentothal. No symptoms of porphyria were evident during the pregnancies and the deliveries were normal. The patient died about one year after the diagnosis of AIP with symptoms of ascending paralysis.

The third case of porphyria in the family was diagnosed in 1962 in a daughter aged 21 years at the time. The first symptoms again were abdominal pain and vomiting, but she also had back-ache. The two years following the diagnosis were almost symptom-free until in November 1964 the patient came to our hospital asking for therapeutic abortion. She complained of increasing back ache but the principal reason she gave to justify her request for abortion was that she did not want to have a child which might have the same disease as she had. She was still unmarried. The patient's general condition was good and no neurological symptoms of porphyria were demonstrable. She was found to be in the 5th or 6th month of gestation. The calculated date of confinement was March 25 1965. Abortion was not considered justifiable. The patient married soon afterwards, and her pregnancy had an uneventful course: only on two occasions, for a few days each, did she have pain in legs and abdomen. She was examined at the Department of Obstetrics on February 11 12 1965 and was found to be subjectively symptom-free. Physical examination gave no abnormal findings. The Ehrlich test revealed porphobilinogen in the urine. The secretion of coproporphyrin per 24 hours was 209 μg and of uroporphyrin 20,800 μg.

On April 12, 1965 the patient was admitted for postmaturity. She was subjectively free from symptoms of porphyria and physical examination again gave no abnormal findings. Her haemoglobin was 10.8 g/100 ml and haematocrit 32 per cent, serum bilirubin 0.9 mg % and serum glutamic oxalacetic transaminase 55 Wroblewsky units per ml. The Ehrlich test was strongly positive. Schlesinger and the iodine test were negative. Oestrogens were administered during the morning of April 13 and regular labour pains started the same evening. A normal delivery followed 36 hours later: the child was a healthy boy, birth weight 3580 g. The placenta weighed 600 g, and histological study revealed placental calcification. The patient was given no drugs during or after parturition. On the day before parturition the urinary secretion of uroporphyrin per 24 hours was 11,590 μg, the day after parturition it was 18,020 μg, and 9 days post partum 9022 μg. Porphobilinogen was also found in the urine on all these occasions. During confinement the patient showed no symptoms suggestive of porphyria. Not until a year post partum

after ingestion of a barbiturate-containing drug for an acute infection, did the patient experience strong abdominal pain which subsided with rest in bed and chlorpromazine

The fourth case of porphyria in the family a girl was treated for mania at the Central Mental Hospital in Oulu at the age of 15 Then she was suspected to have porphyria but porphobilinogen in the urine could only be shown 4 years later in 19 4 in connection with abdominal pain. In 19;6 she contracted paraplegia, but recovered relatively well She is unmarried and has no pregnancies The youngest daughter was diagnosed as having porphyria recently at the age of 17

In addition there is a daughter of 34 who has had two childbirths but no symptoms of porphyria and a daughter of 24 with a history of two child births and a miscarriage In connection with the last delivery she was examined for porphyrins in the urine but none were found.

Acute intermittent porphyria has thus been diagnosed in five daughters of 13 children in the family One of them died of the disease Three of the five daughters with AIP have had a total of nine pregnancies of which six were carried to term two ended with spontaneous abortion and one with induced abortion. Four deliveries occurred before the AIP had been diagnosed, in one case AIP was diagnosed at the beginning of pregnancy and in another case it had manifested itself two years before the pregnancy began. Both spontaneous abortions had occurred before the outbreak of the disease

Discussion

Exacerbations and remissions are common in acute intermittent porphyria. The severity of the attacks varies a great deal even in the same patient. This makes it difficult to assess the effect of pregnancy on its course Reports on accumulated data in the literature usually indicate that pregnancy aggravates porphyria (*Kiss* and *Szemere* 1965 *Seckler* and *Rovinsky* 1965) while in occasional cases pregnancy has been associated with a remission (*O Dwyer* 1955) The series collected by *Seckler* and *Rovinsky* (1965) consisted of 55 women with porphyria they had 48 deliveries before and 67 deliveries after the recognition of the disease. In this series exacerbations occurred in three of four pregnancies while remission occurred only once in every six

pregnancies. In 24 cases the first attack occurred during pregnancy.

The porphyria patients of the family presented had six pregnancies before the outbreak of the disease. In one case AIP manifested itself during the third pregnancy. In this patient the disease was aggravated at the beginning of the next pregnancy which was terminated in the 16th week of gestation and the patient was sterilized. In one patient pregnancy did not affect the course of the porphyria diagnosed two years previously. One patient had four pregnancies, and AIP developed two years after the last pregnancy. It ended fatally after just over a year. On the basis of the present series pregnancy cannot be shown clearly to have provoked the onset of AIP or to have aggravated the disease. The death in the present series was unrelated to pregnancy, whereas in *Seckler's* and *Rovinsky's* series (1965) the mortality rate during pregnancy was 22.4 per cent. On the other hand, it is difficult to attribute this mortality to pregnancy alone, since according to *Waldenström* (1937) the mortality rate within one year of the outbreak of AIP is 20 per cent.

On the basis of the series reported in the literature (*Kiss* and *Szemere* 1965, *Seckler* and *Rovinsky* 1965) it is apparent that exacerbation of porphyria is unusually frequent during pregnancy, some of these exacerbations are fatal. Notwithstanding this, according to *Seckler* and *Rovinsky* (1965) induced abortion is not indicated at least as long as the exacerbation is acute. Twenty three of the 67 pregnancies in their series ended with abortion, and 11 patients died after the abortion, six of these abortions with fatal outcome were spontaneous and five were induced. The present series included one induced abortion after exacerbation of the symptoms of AIP. It was followed by remission. The present authors find that termination of pregnancy should be considered individually in each case, weighing the psychobiological factors which also may be responsible for the exacerbation of AIP (*Watson* 1963).

There is no set rule concerning the method of delivery that should be chosen for patients with acute intermittent porphyria. K[illegible] and *Szemere* (1965) preferred Caesarean section to vaginal delivery, since the moment of delivery can be chosen, the psychic

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excitement associated with delivery can be avoided, and the local anaesthetics of adverse effect, are not required In the present series the deliveries were effected by the normal vaginal route, and no exacerbations occurred. The present authors find that operation is a bigger stress on patient than vaginal delivery and the anaesthetics used are an additional factor (*Seckler* and *Rovinsky* 1965)

Surgical sterilization of AIP patients is considered contra indicated but the patient should be advised to use an efficient contraceptive method (*Seckler* and *Rovinsky* 1965 *Zilliacus* 1967) Today an intrauterine device is probably the most suitable method since oral contraceptive pills are claimed possibly to provoke AIP attacks (*Schmid* 1967) But it has also been claimed that in certain cases oral contraceptives may have prevented AIP attacks (*Perroth et al* 1965)

In conclusion, it seems desirable that women with AIP should not become pregnant. But once pregnancy has started the patient s general condition should be kept as good as possible and drugs provoking attacks such as barbiturates sulphonamides alcohol and ergotamine should be avoided (*Seckler* and *Rovinsky* 1965 *Schmid* 1967) Surgical operations should be avoided during pregnancy unless necessitated by patient s obstetric condition. Chlorpromazine (*Schmid* 1967) is probably the most suitable drug to reduce the symptoms of porphyria. Also cortisone is found to exert a beneficial effect on porphyria during pregnancy (*Zilliacus* 1967) With adequate treatment, delivery can take place in the normal way without exacerbation of the symptoms of porphyria.

SUMMARY

A family with acute intermittent porphyria (AIP) is presented and the possible effect of pregnancy and delivery on the clinical picture is described. The five daughters of the family affected with porphyria, have had a total of nine pregnancies of which six were carried to term, tw ended with spontaneous abortion and one with induced abortion. Pregnancy and childbirth in the present series could not be clearly shown to provoke the onset or exacerbation of AIP during pregnancy

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Table I. *Details of Cases*

Subject			Pregnancy		Cause of Stay in Hospital (H=Healthy Volunteers)
Group	No.	Age (Years)	No.	Estimated Duration (Weeks)	
A.	1	29	II	37	Diffuse pulmonary changes rather miliary discovered at a mother care unit. No definite diagnosis: Scalene fat pad nodes showed nothing abnormal. Lung biopsy specimens revealed granulomatous changes suggestive of tuberculosis but no mycobacteria have ever been found in tissues sputa or gastric washings.
B	1	35	IV	41	Rh-immunization (insignificant)
	2	36	V	42	Social reason
	3	23	II	42	Rh-immunization (insignificant)
	4	30	III	31	H
	5	19	I	41	Suspected postmaturity increased weight
C	1	19	I	38	H
	2	28	I	27	H
	3	26	I	37	H
	4	25	I	32	H
	5	25	II	31	H
	6	24	II	33	H
	7	29	II	29	H
	8	19	I	34	H
	9	27	I	38	H

Case A. Venous blood and thoracic duct lymph sampling only
Cases B. Venous blood sampling before and (Fig. 1) after injection of 10.000 I U of Heparin® (Vitrum) within 15 seconds
Cases C. Capillary sampling only at (within ± 10 minutes) 8 a.m. noon 4 and 8 p.m.

significant amount of the DAO in the bloodstream in men and non-pregnant women after administration of heparin is transported there by the lymph. Assuming the conventional concept of the transport of lymph from the uterus, and a transport of DAO by the lymph, the DAO-activity of the thoracic duct lymph

Hansson R, Tryding N and Törnqvist Å, Acta obst et gynec scandinav 48, 8, 1969

From the Department of Clinical Chemistry (Prof C G Holmberg) University of Lund and the Department of Obstetrics and Gynaecology (Prof S Brody) University of Gothenburg, Sweden

DIAMINE OXIDASE (HISTAMINASE) IN HUMAN PREGNANCY

The Activity of the Enzyme in Lymph and in Blood Plasma and the Effect of Heparin on the Latter

BY

R. HANSSON, N. TRYDING AND Å. TÖRNQVIST

The human placenta contains large amounts of diamine oxidase (DAO) (*Danforth* and *Gorham* 1937 *Ahlmark* 1944 *Swanberg*, 1950 *Kapeller Adler* 1965 *Southren et al* 1965 and 1966)

The increase of this enzyme in the blood during pregnancy (*Marcou et al* 1938) derives at least partly from the decidual cells of the placenta (*Swanberg*, 1950) It appears that no other sources of the DAO in human plasma during pregnancy have been reported in the literature

In human beings intravenous administration of heparin results in an increase of the DAO-activity of the blood plasma to levels otherwise seen only during the latter part of pregnancy (*Tryding*, 1965 a) This effect of heparin varies with the dose given and from one individual to another and appears to be ascribable to a release of DAO into the bloodstream and lymph (*Hansson et al* 1966 *Dahlbäck et al.* 1968)

The DAO-activity of human lymph is normally about 30 times higher than that of the blood and this relation will increase to more than 100 times after administration of 10 000 IU heparin, despite a simultaneous marked rise of the activity in the blood plasma (*Dahlbäck et al* 1968) It would thus appear that a

Diamine: O_2 oxidoreductase (deaminating) E C 1 4.3.6

calculated from a series of n double determinations as $\sqrt{\Sigma \Delta^2 / 2n}$, where Δ is the difference between n duplicate determinations)

Calculation. For each of the nine normal subjects in group C the average value on the day of investigation, $\frac{\Sigma X}{4}$, was estimated and taken as 100 %. The four results were expressed as percentages thereof. The 36 results showed a normal distribution, except for the noon value in patient 9 which was therefore excluded. For each of the nine subjects the standard deviations ($= \sqrt{\Sigma \text{ squares of percentage differences}) / 3}$) and the coefficients of variation $\left(= \frac{SD}{mean}\right) \times 100$ were calculated. Finally the mean value of this coefficient, the standard deviation and standard error of the mean were estimated.

Results. The DAO-activity of thoracic duct lymph from woman Al was 0.50 U/l. The corresponding value for the blood plasma was 1.53 U/l, *i.e.* 3 times as high.

The spontaneous diurnal variation in pregnant women is given in Table II. The variation coefficient for a 12-hour period ranged between 3 and 15 % with a mean of 5 % (±2 %=S.D. A possibly discernible diurnal variation is receiving further attention for later publication)

After heparin. The initial level of the DAO-activity in blood plasma was normal for the stage of pregnancy. The administration of heparin produced no symptoms or signs of any side reactions.

Within 2 minutes of the injection of heparin the two women, who were examined at short intervals showed a plasma-DAO activity which rose to three-fourths of the maximum recorded (Fig. 1). Maximum activity was achieved after approximately 20 minutes in both of the women. In the woman who was examined one hour after a meal the activity curve declined smoothly. For the pregnant woman who had abstained from food and water for more than 12 hours before the test the plasma DAO-activity curve clearly undulated for the first 90 minutes

should be higher than in non pregnant subjects and higher than in the blood plasma For determining the DAO activity of duct lymph and comparing it with that of the blood plasma, lymph was aspirated from the thoracic duct (four weeks before partun tion) in a pregnant woman undergoing left sided biopsy of the scalenus lymph node for diagnosis of pulmonary changes

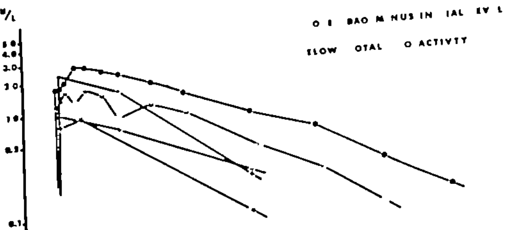
Five women in the last trimester of pregnancy were studied for the effect of pregnancy on the liberation of DAO after intra venous injection of heparin The DAO activity of the blood plasma was determined before and repeatedly during the first few hours after intravenous injection of heparin. Since a search of the literature failed to reveal any investigation of the diurnal variation if any of the DAO-activity of the blood plasma during pregnancy the activity was followed for a 12 hour period in 9 healthy pregnant women.

Clinical Material and Methods

The series comprised patients without any appreciable obstetric complications (see Table I) Heparin® (Vitrum) 5000 IU/ml (cont. tricresol 2 mg/ml) was used *DAO* The diamine oxidase (DAO) activity was determined by Tryding s (1965 b) modifica tion of Okuyama & Kobayashi s method A mixture of 50 μl of the ^{14}C-putrescine 1 mM and 50 μl of plasma or lymph is incubated at 37 C in 1/15 M phosphate buffer pH 7 4 The reaction product, 1 pyrroline but not the putrescine which remains in the water phase is extracted quantitatively by ready prepared scintillator fluid and the ^{14}C activity is counted in a *liquid scintillation* counter *(Packard 2000-series)* One *unit is* defined as the enzyme activity which under the prevailing condi tions of incubation will convert 1 μmole of the substrate per minute

The reaction is of zero order for DAO activity up to 3 U/l if the enzyme concentration is higher shorter incubation or a smaller amount of the sample is chosen

Non-pregnancy values in blood plasma 0–25 mU/l are deter mined with a random error of approximately 20 % whereas normal values from the second or third trimester are determined with a random error of about 1 % (The error of the method was



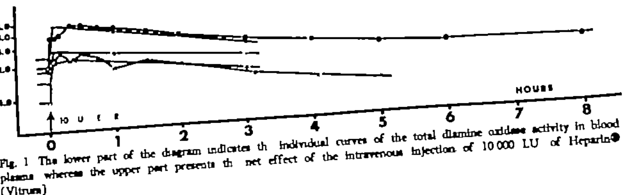


Fig. 1 The lower part of the diagram indicates th individual curves of the total diamine oxidase activity in blood plasma whereas the upper part presents th net effect of the intravenous injection of 10 000 I.U of Heparin® (Vitrum)

Table II *Results*

…bject		DAO Values (U/l) in Blood Plasma (Unless Otherwise Stated)																
…roup	No	Before Heparin				After Heparin (Minutes)												
						2	5	10	20	0	45	60	90	120	180	240	300	360
	1	Lymph. 0.50 blood plasma. 1.53																
	1				1.39		2.06		2.23						1.47			
	2				1.75		2.66					2.40			1.96			
	3				2.17		4.57					3.80			2.54			
	4				.93	2.07	2.23	2.44	2.11	2.46	2.27	1.75	1.98	1.75	1.30	1.14	1.03	–
	5				1.92	3.59	3.60	3.82	4.53	4.51	4.21	4.04	3.61	3.19	2.69	2.44	2.14	2.04
	1	.92	.83	.87	.81	Comments on group C The noon value in subject C9 is not included. If the												
	2	1.98	2.06	1.99	1.90	individual mean value be taken as = 100 % the mean value at 8 a.m is												
	3	1.11	1.18	1.15	1.13	102 % (S.D = 5.7 %)												
	4	.97	.88	.95	.89	noon = 104 % (S.D 5.1 %)												
	5	1.04	1.21	1.00	.97	4 p.m. = 98 % (S.D 4.2 %)												
	6	3.18	2.96	2.79	2.89	8 p.m. = 96 % (S.D 2.9 %)												
	7	1.40	1.58	1.58	1.52	Mean value at 8 p.m. < Mean value at 8 a.m. and noon (0.01 > p > 0.005)												
	8	2.14	2.14	1.84	1.94	If any difference is considered fiducial the percentage values can be accepted												
	9	.65	(.34)	.58	.55	as a normal distribution with S.D = 5.4 %												
…r C	time	8 a.m	noon	4 p.m	8 p.m													

in men and in non-pregnant women of the same age groups (Hansson et al. to be published). This applies particularly to the time of maximum activity which in the 2 women studied most thoroughly occurred about 20 minutes after the injection, compared with 60–120 minutes in men and non-pregnant women. A similar tendency to an early maximum increase of the enzyme was noted also in the other 3 pregnant women, a finding rarely seen in non-pregnant individuals. The heparin-induced increase of the DAO-activity in the blood plasma in the pregnant women did not differ significantly in magnitude from that in non-pregnant women (*Hansson et al.* to be published) but was comparatively large. The slope of the DAO-curve was similar for all the pregnant women, and in the 2 women who were followed longest in the group the curves for enzyme activity approached the original level smoothly.

The increase in the DAO-activity of the blood plasma disappeared with a half time of 70–80 minutes in 2 and at approximately the same rate in the other women in the group. Also in this respect the pregnant women resembled the non-pregnant ones in whom the half time varied between 45 and 90 minutes (*Hansson et al.* to be published).

These half time values differ considerably from the post partum blood plasma findings (*Ahlmark* 1944). Even if the pregnancy initiated histaminase pool that remains in the body after the delivery of the placenta, will retard the decrease in plasma histaminase, the difference found is between a half-time of about 75 minutes in the post-heparin group and a half time of 25 to 30 hours in the post-partum group. This difference would presumably have been greater but for the prolonged lymph infusion, rich in DAO (histaminase) in the post-heparin group (*Hansson et al.* 1966, *Dahlbäck et al.* 1968). If the post-heparin DAO enzyme molecule is identical with the pregnancy-DAO in blood plasma, the different eliminatory rates are an interesting finding. Pregnancy does not markedly influence the elimination of post heparin DAO from plasma. The possibility that there is a puerperal retardation of the elimination of any DAO-activity cannot be ruled out, but the existence of isoenzymes of DAO might also explain the difference.

after the injection of heparin. The subsequent part of the curve was smoother

The "half time of the DAO-differences from the starting level was about 70–80 minutes for both curves. The slopes of the DAO-curve for the other women (each curve was defined by only 2 determinations) showed a half time varying between 40 and 80 minutes

Discussion

Evidence of a significant secretion of histaminase /DAO from the placenta directly into blood vessels (*Swanberg*, 1950) does not exclude the possibility of even a fairly considerable transport of this enzyme via the lymphatics from the uterus (for review of the ileo-pelvic aortic lymphatic system, see *Nelson et al.* 1964) Such a substantial transport via the lymph would imply that the concentration of DAO in the thoracic duct lymph were higher than in non pregnant subjects (for comparison see *Dahlbäck et al* 1968) and higher than in the blood plasma. These two criteria were not observed in the present case No evidence of any congestion of the lymph (owing to tuberculosis for example) was seen, while the woman was in hospital or during a subsequent 12 month follow up. With reservation for the fact that this obstetrically and gynaecologically healthy pregnant woman had pulmonary changes the DAO-activity in the lymph and that in the blood plasma do not suggest that considerable amounts of the enzyme are transported via the thoracic duct

As far as we know the literature contains no numerical data on the diurnal variation of DAO in the plasma in healthy pregnant women. The spontaneous approximately 5 per cent variation around the 24-hour mean found by us showed that the DAO-increase occurring after the injection of heparin is not appreciably influenced by diurnal fluctuations.

The increase in blood plasma DAO following the heparin injection seemingly occurred sooner in the pregnant women than

Control measurement of waste blood (which was taken between sampling to keep the cannula patent) confirmed that the variation in the activity was true

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Control measurement of waste blood (which was taken between sampling to keep the cannula patent) confirmed that th variation in the activity was true

a) In thoracic duct lymph and blood plasma from an obstetrically normal pregnant woman with diffuse granulomatous pulmonary changes the enzyme activity of the lymph was the same as that earlier discribed in men and one non-pregnant woman, while the activity of the blood plasma was 3 times as high as in the lymph i.e. at a level normally found during late pregnancy

b) In 5 healthy women in the third trimester of pregnancy the blood plasma was examined after intravenous injection of 10.000 I.U. heparin (conventional dose in anticoagulant therapy of thrombo-embolism) the DAO-activity rose in a similar manner to the reaction non pregnant women, but possibly somewhat faster and rather higher

c) In the blood plasma from 9 pregnant women, during the daytime spontaneous diurnal fluctuation amounted to about 5 per cent of the mean value.

The investigation produced no evidence of any appreciable transport of DAO from the placenta to the blood plasma via the lymph. The DAO-content of thoracic duct lymph appeared to remain unchanged during pregnancy despite a marked increase of this enzyme in the blood plasma. Neither did the placenta nor the other structures rich in DAO during pregnancy appear to influence significantly the increase in DAO elicited by the heparin.

The spontaneous diurnal variation of the DAO activity in blood plasma during pregnancy will hardly influence the value of a single determination of DAO as a prognostic guide.

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Intravenous injection of heparin in pregnant women thus appears to produce an earlier and possibly but not markedly larger increase of the DAO-activity of the blood plasma than in men and non pregnant women. The activity disappears at approximately the same rate in both pregnant and in non pregnant subjects The high placental content of DAO thus appears to have but little effect on the increase of enzyme in the plasma following intravenous injection of heparin. The reason for this is obscure. It may imply different kinds of DAO enzymes in man but it may also be explained by the assumption that only a relatively small fraction of the heparin injected reaches the placenta and other DAO rich structures within the pregnant uterus (*Southren et al* 1965) Investigation of the effect of intra arterial injection of small amounts of heparin and determination of the DAO activity on the venous side of the uterine vessels appears to be necessary before anything definite can be said about the role played by the placenta in the increase of plasma DAO after injection of heparin in pregnant women. In this respect the problem may be analogous to that of the role of the placenta in the metabolism of exogenous histamine in pregnancy (*Nilsson et al* 1959 and *Lindberg*, 1963)

The DAO activity of the placenta is several thousand units The DAO activity is also high in adjacent structures and has been measured relative to that of the placenta with the aid of ^{14}C-putrescine as substrate (*Southren et al* 1965) Intravenous injection of heparin in a dose of 10–15 000 I U *i e* a concentional single dose in anticoagulant therapy is followed by the liberation of some tens of units of DAO Our investigation thus produced no evidence that the DAO-content of the placenta is influenced by administration of heparin. But even if all the DAO liberated derived from the placenta the local decrease in the concentration of the enzyme would still be only about one per cent or less.

SUMMARY

The activity of diamine oxidase (DAO) /histaminase (E. C 1 4.3 6) was studied in pregnant women and the following observations were made.

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DIAMINE OXIDASE (HISTAMINASE) IN BLISTER FLUID AND BLOOD PLASMA DURING PREGNANCY

BY

ROY HANSSON

The increased histaminolytic activity of human blood plasma during pregnancy (*Marcou et al.* 1938 *Ahlmark* 1944) has been ascribed to the activity of an enzyme histaminase, (*Best* 1929 *Best* and *Mc Henry* 1930) which catalyses the oxidative deamination of among other substrates histamine. It has been discussed (e.g. *Kapeller Adler* 1965 and *Zeller* 1965) whether histaminase really is identical with diaminoxidase (DAO) (*Zeller* 1938) but both names are used synonymously (Report of the Commission on Enzymes of the International Union of Biochemistry Oxford, Pergamon 1961) for diamine oxygen oxidoreductase (deaminating) E.C No 1.4.3.6. The origin of the increase of histaminase in the blood plasma has been traced to the decidual part of the placenta (*Swanberg*, 1950) The relative distribution of the enzyme in human maternal and foetal blood plasma as well as in various trophoblastic and decidual tissues at parturition has recently been studied with the use of a ^{14}C-putrescine method (*Southren et al* 1965) But apart from the report of a single case (*Hansson Tryding* and *Törnqvist* in press) where thoracic duct lymph from a pregnant woman was found to contain approximately the same concentration of DAO activity as that found in non pregnant women and in only one third of the concentration of that found in the woman's blood plasma, little is known about the distribution of the enzyme in the other components of the fluid phase during pregnancy It was recently reported (*Hansson Kiistala Rorsman* and *Tryding*, 1967) that fluid in suction blisters produced by the method of

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Table I

Subject no		1	2	3	4	5
Age (years)		20	21	22	28	33
Pregnancy no		III	I	II	I	IV
Pregnancy week		35	31	28	29	33
Plasma DAO	before	.959	.522	1.11	1.78	2.27
(U/l)	after	.918	.550	1.16	1.82	2.19
	mean	.93	.54	1.14	1.80	2.23
Blister DAO (U/l)		.122	.076	.147	.181	.258
Plasma protein g per 100 ml mean		6.88	6.81	7.24	6.40	6.03
Blister protein g per 100 ml		1.37	1.30	1.48	1.53	1.27
Blister albumin		67.9	60.5	78.4	83.9	71.2
Relative percentage	α_1-globulin	3.6	5.8	3.3	2.4	4.2
	α_2-globulin	4.4	5.8	3.0	2.6	3.8
	β-globulin	9.5	7.7	7.2	7.6	10.1
	β_2-globulin	2.2	3.7	1.5	0.6	3.1
	γ-globulin	12.4	16.5	6.6	2.9	7.6
Suction time before blistering, minutes		124	75	85	150	140

1-pyrroline is almost quantitatively (more than 96 per cent) extracted from the water phase by washing it twice with 10 ml of toluene (in which the scintillators are already dissolved) whereas less than 0.2 per cent of the remaining ^{14}C-putrescine (often less than 0.1 per cent) is contaminant in the resulting 20 ml of scintillator fluid. After each washing the water phase is frozen and the toluene phase decanted into a scintillator vial for counting (Packard Tricarb 2000-series). Sample blanks are prepared in the way described above but not incubated. With an incubation time of 120 minutes and a sample amount of 50 l the response is linear up to an enzyme activity of 3 μmoles of putrescine per minute and litre (i.e. 3 U/l). When more active specimens are measured it is convenient to use a shorter incubation time and/or dilution of the sample. The normal blood plasma DAO-activity ranges from 0 to 25 mU/l in non-pregnant individuals (the same for both sexes). In pregnancy a rise will occur to values between

Kiistala and *Mustakallio* (1964) in non pregnant volunteers contained DAO in a concentration at least as high as that in the blood plasma in spite of the fact that the protein content was only about one third of that in the latter Injection of heparin was followed by an increase of DAO but the increase was only a fraction of that which occurred in the blood plasma

In order to assess the possibility of ultrafiltration of DAO from the blood plasma into suction blisters the protein content and enzyme activity of the fluid from such blisters in pregnant women were determined and compared with the corresponding concentrations in the plasma

Material and Methods

Samples of venous blood were obtained from 5 healthy non fasting pregnant women immediately before the test. Further information on the volunteers is given in Table I The blisters were produced by the method of *Kiistala* and *Mustakallio* 1964 The proximal area of the skin on the volar aspect of the (left) lower arm was washed with diluted alcohol and allowed to dry in the air The suction cup of the angiosterrometer was applied. A negative pressure of 200–250 mm Hg was produced and maintained long enough to produce blisters from which altogether about 200 μl of fluid could be obtained The negative pressure was then gradually diminished and the cup and the apparatus were removed. With a thin sterile cannula and a plastic syringe the blisters were punctured and their content was transferred to a microcentrifuge tube made of polypropylene (Sarsz model) Samples of venous blood were obtained immediately before and after the blisters had been emptied.

The DAO-activity was estimated according to the principles of *Okuyama* and *Kobayashi* (1961) in a modification for ultra micro-samples by *Tryding* (1965) ^{14}C putrescine SA 1 6 Ci/mole 1 mM, in 1/15 M phosphate buffer pH 7 4 and an equal volume (50 μl) of blister fluid or blood plasma are incubated in 1 90 ml 1/15 M phosphate buffer pH 7 4 at 37 C for 120 minutes and, thereafter immediately chilled to about 0 C by immersion in a cold (−15 C) bath The reaction product, ^{14}C

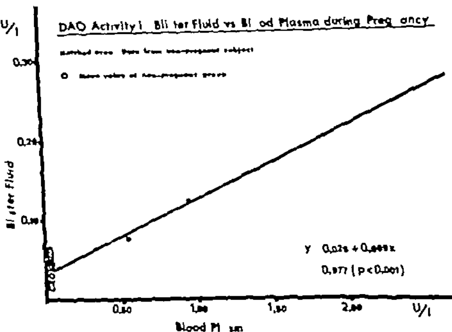


Fig. 1.

Discussion

The blisters that form under the suction cup of the angiosterrometer have no direct point of contact with the apparatus. In our series the longest interval between formation of the blister and puncture was 30 minutes. The concentration of the sodium and that of the potassium in the blister fluid were identical with the normal concentration in plasma/intercellular fluid. This suggests that the cell membranes had not been damaged, but it does not exclude the possibility of a very rapid electrolyte shift between the blister and interstitial fluids being able to eliminate any differences that might have occurred. No appreciable difference was found in the blistering tendency among the pregnant women, compared with previously examined non-pregnant volunteers. The concentration of the protein in the blisters was fairly uniform in all the women. In the previous investigation (*Hansson*

0 9 and 8 U/l. The coefficient of variation of the procedure, as calculated from a series of double determinations with Dahlberg s formula (S.D = $\sqrt{J^2 \quad (2n)}$ ¹ and coeff var = S.D (mean value of series) [1]) is between 15 and 30 per cent in the lower normal range of DAO-activity and in the pregnant group less than 2 per cent.

The protein concentration in the blister fluid was determined by the *Waddell* method (1956) and in blood plasma by the biuret method. Paper electrophoresis was performed by the method of *Laurell Laurell* and *Skoog* (1956) The alkaline cations were determined by flame photometry *(Eppendorf)* in the pooled surplus blister fluid (in 10 to 40 μl portions)

Results (see Table I)

The mean protein content of the blood plasma was found to be 6 7 (Range 6 0–7 2) and that of the fluid from the blister 1 4 (Range 1 3–1.5) g per 100 ml. Paper electrophoretic separation of the blister content showed the following pattern

Albumin	72.4 % (Range 60 4–83.9) of the protein
α_1-globulin	3 9 % (Range 2.4–5.8) "
α_2-globulin	3.9 % (Range 2.6–5.8) "
β_1-globulin	8.4 % (Range 7.2–10 1)
β_2-globulin	2.2 % (Range 0 6–3 7)
γ-globulin	9 2 % (Range 2.9–16.5) "

The concentrations of sodium and of potassium in the blister fluid (triplicate determinations) were 140 and 4 55 mEq/l respectively The DAO-concentration in the blood plasma at the beginning of the test did not differ significantly from that found at the end of the test.

The concentration of DAO in the blister fluid v as positively correlated with the corresponding activity in the blood plasma (Fig 1)

In two volunteers studied, the blister content together with an equal amount of blood plasma was found to possess the same enzyme activity as the sum of the activities in the individual samples determined separately DAO-inhibitor could thus not be demonstrated in the blister fluid as compared with blood plasma.

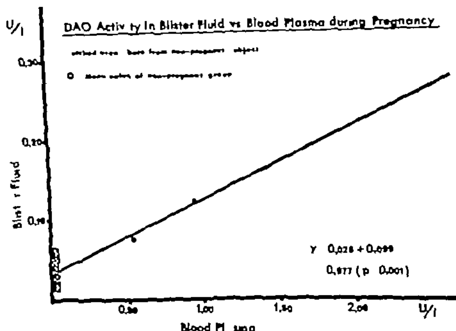


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et al 1967) the protein concentration varied but in that series the subjects differed in age and the application site of the suction cup and the total size of the blisters varied much more than in the present series The intercellular fluid is not synonymous with lymph from the same area especially regarding the concentration of protein, which in the lymph vessels has been found to be higher than in the extravascular interstitial space (*Földi* 1967) The concentration of protein in blister fluid is somewhat lower than that given for the interstitial fluid of the dermis (*Schulte* and *Heremans* 1966) but higher than that in oedematous fluid in cardiac oedema and is of the same order as in lymphoedematous interstitial fluid. The paper electrophoretic pattern suggested only a protein-containing ultrafiltrate and did not differ significantly (Student s t test) from findings in non pregnant women (*Hansson et al* 1967) Therefore it would appear that pregnancy is not accompanied by any appreciable change in the relative composition of the protein in blister fluid. The DAO activity of blister fluid proved to be clearly dependent on the concentration of the enzyme in the blood plasma. The regression line intersected the normal range (Fig 1 hatched area, *Hansson et al* 1967) near the mean value found for non pregnant women. The DAO-content of the blister fluid may thus have a component not dependent on the DAO-content of the blood plasma and secondly a component of much lower concentration than, but correlated with that in the blood plasma In non pregnant individuals the activity of DAO was on the average about 1 7 times as high in blister fluid as in plasma and much higher (about 5 times) if the enzyme activity was correlated with the protein concentration (*Hansson et al* 1967) The plasma-correlated component of DAO was about 10 per cent of that in the plasma in our comparatively small group of volunteers If the activity of this enzyme in blister fluid were correlated with the protein concentration and the same manoeuvre performed with DAO and protein concentrations in plasma the resulting specific activities in the two fluids would be more similar The relative DAO-content of the blister fluid then was about half of that in the plasma

The suction blister and its fluid are an artefact. The electrolyte

concentration, protein content and electrophoretic pattern suggest that the negative pressure sucks the fluid (interstitial fluid and lymph?) out of the dermis without any appreciable injury to the cell membranes. An alternative or associated explanation is that the fluid leaves the blood stream by ultrafiltration.

In a previous study it was found that thoracic duct lymph from a pregnant woman contained 0.5 U DAO/l, while the blood plasma contained DAO in a concentration 3 times higher (*Hansson Tryding* and *Törnqvist* in press). Since the concentration of the enzyme in the lymph was of the same order as in men and a non-pregnant woman (*Dahlbäck et al.* 1968) this finding was thought to suggest that the *lymph* vessels do not play any appreciable role in the transport of DAO into the blood stream in pregnant women. Neither does the interstitial fluid and lymph as a "DAO-compartment" appear to have any great quantitative importance during pregnancy.

Our present findings indicate that the tendency of DAO to be ultrafiltrated out of the blood stream is relatively small. In the literature the DAO is usually described as an enzyme activity but no definite information is available about its molecular size or about its isoenzymes if any. Therefore our findings do not warrant any conclusions regarding the ultrafiltrability of the enzyme as a manifestation of the behaviour of a homogenous molecule. One might, however, imagine the possibility of one component formed by or liberated from the local tissue (distally to or *immediately* under the suction cup). The behaviour of the second component requires simultaneous examination of lymph or interstitial tissue from the limbs and of blister fluid to find out whether ultrafiltration occurred in situ or whether a small increase of DAO due to the increase in the plasma during pregnancy really occurs also in the interstitial fluid.

SUMMARY

During pregnancy blister fluid, obtained by the technique of *Mustala* and *Mustakallio* shows the same protein pattern after paper electrophoretic separation as in non-pregnant individuals.

The diamine oxidase (DAO)-activity however is higher than

in non pregnant persons and shows a positive correlation with the plasma DAO activity The enzyme activity is about one tenth of that in plasma But without known correlation to the plasma enzyme level there seems to be some blister fluid DAO-activity of the same magnitude as in non pregnant individuals. The findings do not indicate that during pregnancy the extra vascular intercellular compartments store large amounts of DAO as compared with the intrauterine structures and the blood plasma

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THE ¹³¹I TRIIODOTHYRONINE-RESIN UPTAKE TEST DURING LABOUR

BY

O. CASTRÉN, L. LAAKSO AND T. NIKKARI

The size of the unsaturated portion of plasma proteins binding the thyroid hormones (mainly thyroxine-binding globulin, *i.e.* TBG) can be studied by means of the ^{131}I triiodothyronine uptake test (T_3-test) the smaller the uptake the larger the unsaturated portion, and *vice versa*. Since the experiment is performed *in vitro* it can also be used to examine the thyroid status during pregnancy without detriment to the foetus. Assessment of the results, however is difficult, because the size of the unsaturated portion of TBG depends both on the thyroid hormone content and on the amount of TBG itself (*Sisson* 1965). Low T_3-uptake test values are seen in hypothyroidism, the amount of thyroid hormones in the plasma being reduced. During pregnancy the T_3-uptake drops apparently as a result of the increased total capacity of TBG (*Robbins* and *Rall* 1960) the PBI increases for the same reason (*Hamolsky et al.* 1959). An increase of TBG is seen 3–4 weeks after ovulation (*Dowling et al.* 1956 *Hamolsky et al.* 1959) as is the fall in T_3-uptake. The T_3-uptake is at its lowest level in the 2nd to 3rd trimester when it is 60–80 per cent of the corresponding value of the non-pregnant woman (*Spafford et al.* 1960 *Clark* and *Horn* 1965). Its restoration to the normal level occurs one to two weeks *post partum* (*Hamolsky et al.* 1959) although the TBG does not reach its normal level until the fifth *post partum* week (*Dowling et al.* 1956). Preliminary studies showed that changes also occur in the red cell

uptake of ^{131}I labelled T_3 during labour (*Laakso* 1964) The purpose of the present study was to shed additional light on these changes.

Material

The series comprised 15 parturients with no symptoms of toxaemia of late pregnancy Venous blood samples were drawn into heparinised test tubes as follows

A = 7 days before the calculated term,
B = on the parturient s admission to hospital, when labour had started but contractions were still irregular
C = during labour the uterine os 5–7 cm open,
D = immediately *post partum* before expulsion of the placenta,
E = 2–3 hours *post partum*
F = 3 days *post partum*
G = 5 days *post partum*
H = from the umbilical cord at the moment of division.

Caesarean section were performed in cases 14 and 15 because of contracted pelvis. At the time of operation, the contractions were still irregular and the uterine os 2–4 cm open.

The control series consisted of five euthyroid non-pregnant women of fertile age their blood samples were taken in the pre-menstruum.

Method

The resin T_3-uptake was measured using the method of *Woldring et al* (1961) The plasma was separated from the cells by centrifugation. To 20 mg of the resin (Amberlite CG-50 type II) in 2 cc physiological saline solution was added 0 1 cc radioactive ^{131}I triiodothyronine (T_3) (about 0 01 μg) and 0.5 cc of the plasma to be investigated. The mixture was incubated in a water bath at 37 C for 30 minutes

After incubation, the total radioactivity was measured in a well type scintillation counter The tubes were then centrifuged supernatant liquid removed and the resin washed and recentri fuged five times with saline solution. After these washings the tubes were recounted to determine the resin uptake of radio-

Table I. T_3-uptake ratios (multiplied by 100) of plasma samples taken A. before labour B during irregular uterine contractions C. during labour (in cases 14 and 15 immediately before operation) D immediately post partum, E–G 2 3 hrs 3 and 5 days post partum respectively H from the cord blood

Patient	A	B	C	D	E	F	G	H
1		74	83	73	72	89		116
2		74	78	76	54	78		
3			90	87	93	102		103
4		89	92	85	78	98		132
5		74	67	60	72	84		89
6		62	83	56	54	56		87
7		75	88	78	60	74		92
8	84		94	85	73	79	88	108
9	78		78	78	80	83	88	96
10	77	80	82	77	74	75	90	102
11	74		73	80	79	78	82	88
12	67	76	86	79	74	83	87	94
13	67	70	74			72	74	
	6	9	13	12	12	13	6	11
M	75	75	82	76	72	81	85	101
SD	±7.9	±7.0	±8.0	±9.4	±11.2	±11.6	±5.9	±13.8
SEM	±2.9	±2.3	±2.2	±2.7	±3.2	±3.2	±2.3	±4.1
14		88	95	84		101		
15		85	115	91		100		

Abbreviations n. number of samples M mean, SD standard deviation, SEM standard error of the mean

activity The uptake was calculated as a percentage of the original radioactivity (= T_3-uptake percentage) To render the values obtained at different times comparable with each other the uptakes were also expressed as a ratio to the simultaneous resin uptake of the control sera (resin uptake ratio Clark and Horn 1965)

Results

The results are presented in Table I It is seen that the T_3-uptake ratio befor and during labour was significantly lower than in

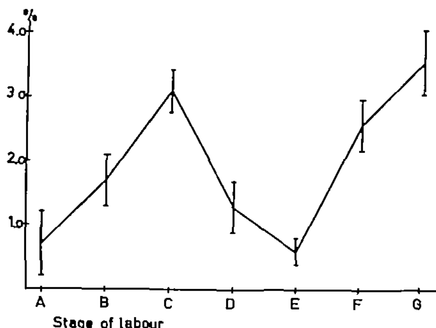


Fig 1 Mean difference of the T_3-uptake percentages at various stages of labour from the minimum T_3-uptake values The standard deviations of the differences are given by vertical segments

the non pregnant women ($p < 0.001$) A week before the cal culated term and as long as the contractions were irregular (A–B) the T_3-uptake ratio was only about 75 per cent of the control value By the time the contractions were regular and the uterine os was 5–7 cm open (C) the T_3-uptake ratio increased to an average of 82 per cent, to fall again, in the sample drawn im mediately *post partum* (D) to 76 per cent of the control value the lowest value 72 per cent of the control value was obtained in the present study 2–3 hours *post partum* (E) Subsequently the T_3-uptake ratio increased gradually although on the fifth *post partum* day (G) it was still 15 per cent lower than the control value

Since the T_3-uptake was found to fluctuate during labour the authors wished to discover whether the changes might be attri butable to the strain produced by labour or to the contractions of the uterine muscle To exclude these factors samples were drawn from two patients who because of a contracted pelvis

had to be treated by Caesarean section in an early phase of labour (Cases 14 and 15) The mean resin uptake ratios of these patients in the different phases of parturition (Table I) were found to be as follows B 88 C 105 D 87 F 100 per cent. These changes, although not so marked were parallel to those found during normal labour

The mean T_3-uptake of the blood sample taken from the umbilical cord was the same as in the control series.

To obtain a better idea of the changes in T_3-uptake in the course of labour the lowest percentage obtained for each parturient (most frequently at stages D–E) was subtracted from the T_3-uptake percentages obtained in the various stages of labour Fig. 1 shows the resulting mean differences from the minimum values. It should be noted that the time intervals, seemingly equal on the abscissa, were not in reality of equal duration. The T_3-uptake so calculated was found to increase from A to C highly significantly ($p < 0.01$) to fall from C to E very highly significantly ($p < 0.001$) and to increase again from parturition to the fifth *post partum* day very highly significantly ($p < 0.001$) Consequently the changes in the course of labour cannot be considered accidental they must have been produced by some factor associated with the labour

Discussion

In agreement with earlier findings the present study showed that the T_3-uptake towards the end of pregnancy was only 75 per cent of the values obtained on non-pregnant women, and the normal value had still not been reached five days *post partum*.

No attention has been given previously to the changes in T_3-uptake during labour The rise and fall of the T_3-uptake recorded in the present study in the course of a few hours and in conjunction with parturition, are difficult to explain. The T_3-uptake is known to be increased in hyperthyroidism, in which the ratio of thyroid hormone and TBG is elevated. During pregnancy the capacity of serum binding proteins, especially TBG is increased, but no changes in these parameters have been shown during labour Thyroxin crosses the placenta (*Pickering et al.* 1958)

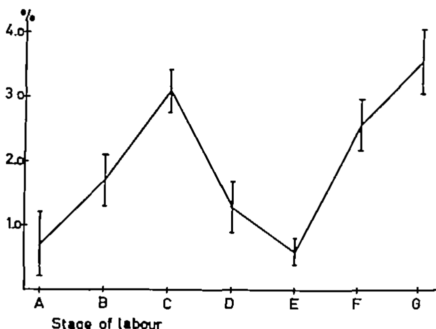


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thereafter slowly (81 per cent on the 3rd and 85 per cent on the 5th day *post partum*).

In samples of umbilical cord blood the mean T_3-uptake was 101 per cent of the value for the non-pregnant women.

The factors hitherto known to change the capacity of the serum proteins to bind thyroid hormone, and therefore also the T_3-uptake, were discussed. The rapid changes found in the present study could not be explained on the basis of such factors alone.

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and high free thyroxin values have been recorded in the newborn (*Marks* 1965) The T_3-uptake of the newborn, however has been reported to be reduced (*Spafford et al* 1960) or increased (*Marks et al* 1961) or as in the present study it has been found to be normal In any case foetal changes are hardly likely to be sufficient to account for the maternal variations

In nephrotic patients the T_3-uptake increases because there is a loss of proteins including TBG (*Robbins* and *Rall* 1960)

Administration of oestrogens reduces the T_3-uptake (*Engbring* and *Engström* 1959 *Dowling et al* 1960) a finding that might explain the low values recorded during pregnancy Similarly chorionic gonadotropin secreted after hysterectomy by chorionic carcinoma metastases reduces the T_3-uptake (*Godden* and *Garnett* 1964) Steroids inhibiting ovulation have the same effect (*Hollander et al* 1963) which is also demonstrable in the premenstruum (*Horster* and *Klein* 1964)

The changes listed above in maternal or foetal thyroid function or perhaps in placental function during labour and the accompanying changes in oestrogen or gonadotropin secretion can hardly account for the relatively rapid changes found in T_3-uptake during labour It is apparent that the binding sites of thyroid binding protein are transiently reduced in the course of parturition for a reason to be ascertained by later studies

SUMMARY

T_3-uptake tests were performed on 15 normal parturitients The samples were taken one week before the calculated term in different stages of labour and *post partum*. A series of euthyroid non pregnant women acted as controls The T_3-uptake at the end of pregnancy just before parturition was 75 per cent of the control level

While the contractions were regular during labour and when the uterine os was open, the T_3-uptake rose to 82 per cent of the control values and fell to the pregnancy level in the sample taken immediately *post partum*. The uptake was at its lowest 2–3 hours *post partum* (72 per cent of the control level) and increased

3.000–4.000 g) and that of the controls 3.840 g (range 3 040–5 150 g) The experimental conditions were the same as in earlier studies (Gelli et al. 1966 and 1968) Thirteen rabbits were given an i.v infusion of 30 % glucose solution in the course of 6 hours on the 29th day of pregnancy The infusion was given through a polyethylene catheter introduced into an auricular vein and ad vanced to the vena cava Shortly after the infusion, the mother was sacrificed by intracisternal injection of 2 ml of a 2 % Xylocaine® solution The foetuses were removed via laparotomy One foetus was placed in a bath of liquid paraffin (37 C) and the ECG recorded. The remaining foetuses in each litter were placed with intact membranes in a thermostat bath (37 C) of physiological saline they were thus made anoxic. As long as heart activity could be recorded by ECG (Gelli and Gyulai 1968) foetuses were taken at irregular intervals for biochemical analyses. Before sampling, the occurrence of heart activity was checked.

The controls consisted of foetuses whose mother had not been given a glucose infusion but were otherwise treated identically to those of glucose-loaded mothers. As a rule, one litter from a glucose-loaded mother and one from an untreated mother were studied concurrently

Foetal blood was obtained by heart puncture. The blood was carefully and slowly aspirated in order to avoid formation of bubbles and haemolysis. It was then transferred to a microlitre test tube which was centrifuged for 2 minutes at 15 000 r p.m. The plasma was separated off and kept deep-frozen for electrolyte determinations. A separate blood sample was taken for determination of lactate This blood was precipitated within 20 seconds with 30 % trichloroacetic acid (TCA) and the lactate determined according to *Barker* and *Summerson* (1941) Micromethods were used for determination of blood pH, total CO_2, potassium, chloride calcium, total protein inorganic phosphorus and lactate (*Sanz* 1957 and 1962 *Thalme* 1962, 1963 1964 and 1967)

Samples from the apex of the heart were taken for electrolyte determinations. Potassium and magnesium were determined with atomic absorption flame photometry (*Berowlede Bergström* and *Hultman* 1968) The muscle samples were dried at 90 C and

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HEART MUSCLE AND PLASMA ELECTROLYTES IN NORMAL AND GLUCOSE LOADED RABBIT FOETUSES UNDER ANOXIA

BY

M. G GELLI, J BERGSTRÖM, E HULTMAN AND B. THALME

In earlier studies in rabbit foetuses we showed that intravenous infusion of glucose in the mother on the 29th day of pregnancy raised the glycogen content of the heart in the foetuses and that their heart activity had a longer duration under anoxia than that in control foetuses whose mother had not received glucose (*Gelli et al* 1966 and 1968) Of all the parameters measured—*i.e* heart muscle glycogen liver glycogen blood glucose and blood pH—the raised heart muscle glycogen content appeared to be the only factor that could explain the prolongation of heart activity It is however known that the electrolyte conditions both extra and intracellularly have a great influence on the heart activity (*Rona Kahn* and *Chappel* 1965 *Selye* and *Gabbiani* 1965) It is also known that the cells lose potassium to some extent under anoxia (*Calkins Taylor* and *Hastings* 1954)

The object of the present experiments was to define in greater detail the influence of these factors

Material and Methods

The material consisted of 28 pregnant rabbits of local breed the average weight of the experimental animals was 3 730 g (range

tein concentration using the following formula (*Eisenman Mac kenzie* and *Peters*, 1936)

$[H_2O] = 984 - 7.18$ protein concentration (g/100 ml plasma)

Excess sodium (Na) *i.e.* the quantity of Na not present in the chloride space was calculated as follows

$$Na = Na_m - \frac{[Na]\ H_2O_{Cl}}{1000}$$

where Na_m = the sodium content of muscle in mEq/100 g FFS and [Na] = the extracellular (plasma) sodium concentration in mEq/L.

Similar calculations were made of K_x i.e. the quantity of K present outside the chloride space in the muscle. H_2O, *i.e.*, the quantity of water not present in the chloride space was calculated as $H_2O_m - H_2O_{Cl}$.

It is true that the chloride space cannot be regarded as a satisfactory measure of the quantity of extracellular water since varying amounts of chloride are present intracellularly in different cells. The real extracellular quantity of water in muscle must therefore be less than the chloride space. We nevertheless calculated the size of this space and used it as the highest possible value of extracellular water. This made it possible to calculate a highest possible value for the extracellular proportion of a given electrolyte in muscle and consequently by subtraction of this amount from the total tissue content, a lowest possible value for the intracellular quantity of the relevant electrolyte (denoted here as excess K (K_x) excess Na (Na) etc.) If an increase in a certain electrolyte *e.g.* K is considerably greater than the maximally possible extracellular proportion—*i.e.*, there is a small difference between total K and excess K —one can therefore conclude that an intracellular increase has occurred. Excess water was interpreted according to similar principles.

Results

Plasma electrolytes

The individual values of plasma pH, K lactate Na Cl PO_4 Ca protein and total CO_2 in the controls and experimental foetuses during anoxia are given in Figs. 1–8

the fat extracted with petroleum ether In 5 experimental and 5 control litters duplicate muscle samples were weighed on a Cahn electrobalance both before and after drying, as well as after ex traction of fat using a method previously described (*Bergström* 1962) and the water content was determined

In the cases where duplicate samples were obtained one sample was taken for determination of sodium, chloride potassium and phosphorus by neutron activation analysis (*Bergström* 1962) The neutron irradiations were performed in reactor R 1 at AB Atomenergi Stockholm The samples from one litter were generally irradiated together as close as possible to reduce the influence of neutron flux variations. Good agreement was found between duplicate potassium determinations with activation analysis and absorption flame photometry

Samples for lactate determination were taken from 12 litters. These muscle samples were frozen directly in liquid nitrogen and then freeze-dried. The freeze-dried sample was analyzed with an enzymatic method (*Scholz et al* 1959) The results were ex pressed in mmol/100 g dry weight.

The values obtained for water and electrolytes were expressed with 100 g fat free substance (FFS) as reference base

The chloride space *i e* the distribution volume for chloride under the assumption that the chloride concentration in this space is the same as in the extracellular fluid was calculated according to the following formula

$$H_2O_{Cl} = \frac{[Cl]_m \cdot 1000}{[Cl]}$$

where H_2O_{Cl} = the chloride space in ml $[Cl]_m$ = the chloride con tent in mEq/100 g FFS and $[Cl]$ = the extracellular chloride concentration in mEq/l

The extracellular chloride concentration was calculated from the plasma chloride concentration $[Cl]_P$ and plasma water $[H_2O]_P$ and a Donnan factor of 0.96 according to the following formula

$$[Cl] = \frac{[Cl]_P \cdot 1000}{0.96 \cdot [H_2O]_P}$$

The plasma water (ml/l plasma) was calculated from the pro-

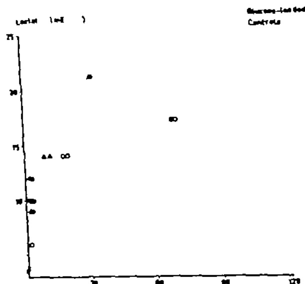


Fig. 3. Blood lactate during anoxia.

pH (Fig. 1) Blood pH fell successively in both groups. The values in the glucose-loaded foetuses tended to be somewhat lower than in the controls at the end of anoxia.

K (Fig. 2) A rise in the plasma potassium occurred in both groups, and was significantly higher in the control foetuses than in the glucose-loaded foetuses in the intervals 0–10 min ($P < 0.01$) 11–30 min ($P < 0.01$) and 31–50 min ($P < 0.01$).

Lactate (Fig. 3) The lactate concentration increased greatly in both groups. The main increase occurred during the first 50 minutes. There was a significant difference between the mean values obtained at 0–10 min and 50–70 min ($P < 0.001$).

Na and Cl (Fig. 4) The levels of sodium and chloride remained fairly stable during the time of the experiment.

PO (Fig. 5) During anoxia the concentration of inorganic phosphorus in plasma rose successively. The increase was significant ($P < 0.001$) with no difference between the groups.

Ca (Fig. 6) The plasma calcium remained fairly unchanged in both groups during anoxia.

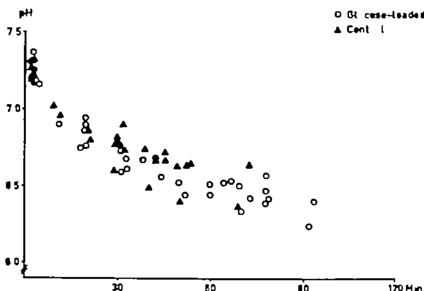


Fig. 1 Blood pH during anoxia.

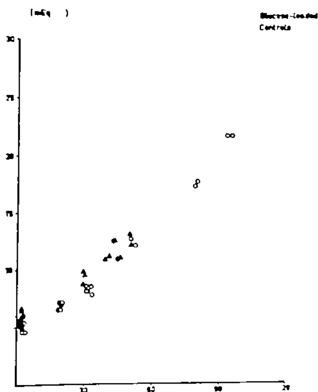


Fig. 2. Plasma potassium during anoxia.

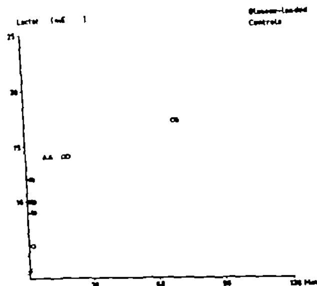


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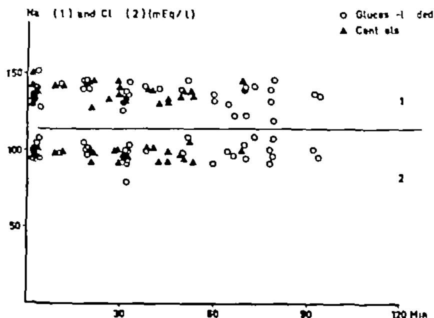


Fig. 4. Plasma sodium and chloride during anoxia.

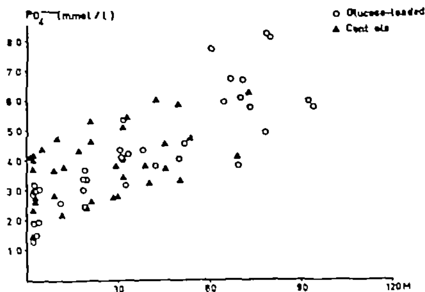


Fig. 5. Inorganic phosphorus in plasma during anoxia.

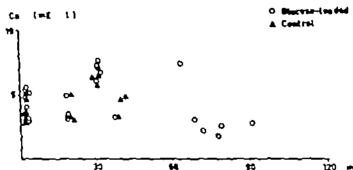


Fig. 6. Plasma calcium during anoxia.

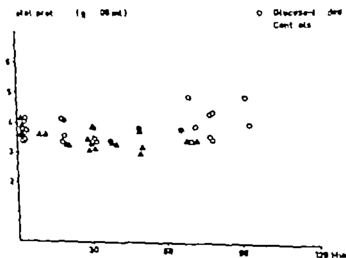


Fig. 7 Plasma protein during anoxia.

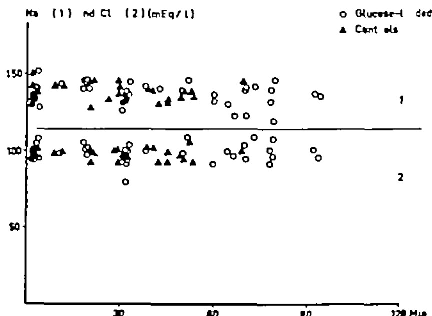


Fig. 4. Plasma sodium and chloride during anoxia.

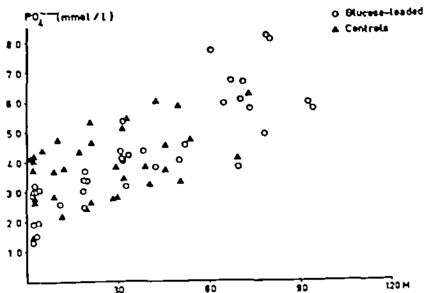


Fig. 5. Inorganic phosphorus in plasma during anoxia.

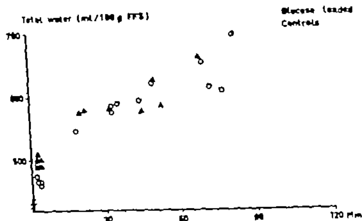


Fig. 9 Total water in heart muscle during anoxia (FFS = fat-free solids).

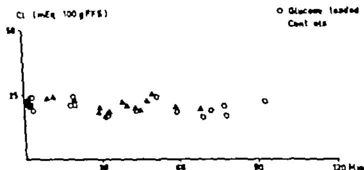


Fig. 10 Chloride content of the heart muscle during anoxia.

Excess water (H_2O_e) (Fig. 11) This showed a marked rise according to the same pattern as the increase in total water.

K (Fig. 12) and K_e. During anoxia, a marked increase took place in both total K and excess K (K_e). The rise in potassium content was most rapid during the first 30 minutes. It showed the same course in both groups, *i.e.* no significant difference was present when foetuses from both groups were in-

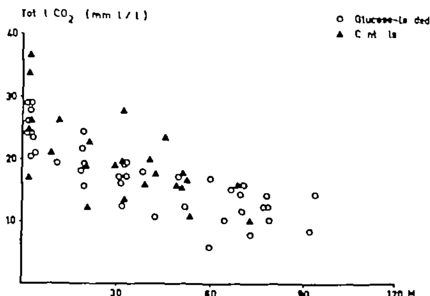


Fig 8 Foetal carbon dioxide in plasma.

Total protein (Fig 7) The concentration of total protein remained fairly stable and of the same magnitude in both groups

Total CO_2 (Fig 8) The total CO_2 fell successively during anoxia in both groups

Muscle electrolytes (Table I)

The mean and range of H_2O Cl H_2O_{Cl} H_2O K K lactate Na Na P the K /P ratio and Mg are given in Table I The individual values are given in Figs 9–15

H_2O (Fig 9) The water content was initially almost significantly higher in the control group than in the experimental one ($P < 0.05$) The content rose greatly in both groups The main increase took place during the first 30–40 min After 30 minutes no significant difference is present between the groups.

Cl (Fig 10) No significant change in the total chloride content occurred in either group

H_2O_{Cl} In similarity to the total chloride the chloride space underwent no significant change in either group

K mEq 100 g FTS	range	47.5–53.2	45.6–47.2	58.2–61.5	53.6–61.3	59.9–71.7	60.6–69.2	61.0–67.4	63.5–77.4		68.7–85.9	
	$\bar{x}$	49.5	46.7	59.8	8.0	65.7	66.4	64.7	70.4		77.7	75.8
	n_1	5	5	5	3	7	6	4	3		4	1
	n_2	5	5	3	3	5	5	4	2		4	
K_i mEq 100 g FTS	range	44.7–46.8	46.3–52.0	51.7 56.7	54.2–60.1	57.7–69.1	58.6–67.6	60.2–64.4	60.2 78.3		66.0–82.2	
	$\bar{x}$	45.7	49.4	54.3	46.6	63.0	64.8	62.5	66.9		74.5	71.0
	n_1	5	5	5	3	7	6	4	3		4	1
	n_2	5	5	3	3	5	5	3	2		4	
Lactat mmol/100 g dry weight	range	8.1–22.4	6.9–16.6	19.0–38.9	25.4–28.1	27.5–54.3	36.3–48.8	39.4–56.3	43.0–64.0	48.0–48.7	45.9–76.1	
	$\bar{x}$	14.2	13.0	27.3	26.8	44.1	42.2	47.9	56.2	49.4	60.7	
	n_1	4	7	5	4	7	9	6	7	2	7	
	n_2	4	7	4	2	4	7	2	5	2	6	
N mEq/100 g FTS	range	23.9–32.6	23.3–28.6	24.4–29.5	24.8–32.2	27.1 32.9	23.8–28.2	27.9–36.3	25.2–29.0		24.2 29.4	
	$\bar{x}$	27.9	25.5	27.4	30.0	29.6	26.6	31.2	27.1		26.1	29.2
	n_1	5	5	5	3	7	6	4	4		4	1
	n_2	5	5	3	3	5	5	3	2		4	
Na mEq/100 g	range	−4.6–2.1	3.4–0.9	1.1 1.9	−0.7–0.4	1.1–6.0	−0.3–5.5	1.8–6.5	4.4–4.6		0.8–5.1	
	$\bar{x}$	1.3	1.0	0.3	−0.1	3.9	2.4	3.5	4.5		2.8	0.6
	n_1	5	5	5	3	7	5	4	3		4	1
	n_2	5	5	3	3	5	5	3	2		4	
P mmol/100 g FTS	range	41.6–48.2	41.7–46.2	41.9–47.1	42.1–45.6	42.5–47.0	42.1–47.3	45.0–49.4	43.0–45.9		44.5–49.5	
	$\bar{x}$	44.4	43.5	44.2	44.3	44.7	44.5	47.2	44.5		46.7	44.6
	n_1	5	5	5	3	7	6	4	3		4	1
	n_2	5	5	3	3	5	5	3	2		4	

Table I *Water and Electrolytes in Heart Muscle*

oxia (min)		0–10		11–30		31–50		51–70		71–90		91–110	
tuses		Contr	Gluc	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc
O	range	479–509	459–494	567–589	531–560	580–703	573–625	583–641	567–646		605–698		
/100 g	x̄	496	472	578	546	629	592	615	607		652		667
S	n_1	5	5	4	3	6	6	4	3		4		1
	n_2	5	5	3	3	5	5	3	2		4		
	range	20.9–23 4	18.9–22 8	19.2–21 7	20.3–24 1	18.6–22.4	16 1–19.9	19.9–22.2	17 4–30 0		16.5–19.9		
Eq/100 g	x̄	21 8	21 1	20 6	21.6	20.5	17.8	20.8	23 7		18.7		21.8
FS	n_1	5	5	5	3	7	6	4	3		4		1
	n_2	5	5	3	3	5	5	3	2		4		
$_2O_{Cl}$	range	197 213	185–201	190–208	177–219	186–211	146–207	186–213	153–171		158–183		
l/100 g	x̄	204	191	197	196	192	171	201	162		172		212
FS	n_1	5	5	5	3	7	6	4	3		4		1
	n_2	5	5	3	3	5	5	3	2		4		
I_2O	range	276–318	263–314	385–388	304–370	395–487	377–487	391–425	410–481		417–535		
nl/100 g	x	295	280	387	346	432	419	414	446		487		434
FS	n_1	5	5	5	3	7	6	4	3		4		1
	n_2	5	5	3	3	5	5	3	2		4		
K	range	45.5–53 8	44 4–51.2	52.1–62.6	51 7–62.7	62.0–72.9	63.3–71.8	54.9–74.5	63.6–77.0		68 7–80 4		
mEq/100 g	x̄	50 3	48.2	58.5	56 7	65 9	67 4	68.0	70.6		75.6		80 4
TTS	n_1	6	9	8	10	11	11	6	3		7		1
	n_2	6	9	5	7	6	9	4	2		7		

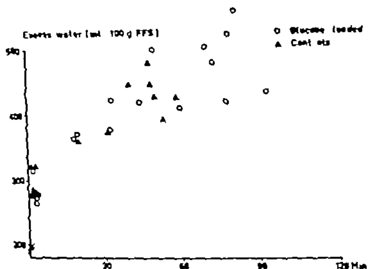


Fig. 11 Excess water in heart muscle during anoxia.

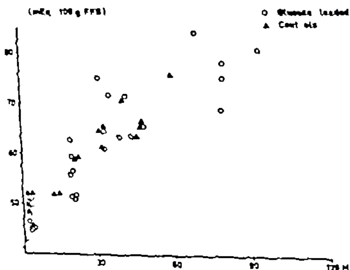


Fig. 12 Potassium in heart muscle during anoxia.

Table I (continued)

Anoxia (min)		0–10		11–30		31–50		51–70		71–90		91–110	
Foetuses		Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.
K /P ratio	range	1 1–1.2	1.0–1.2	1.3–1 4	1.2–1.5	1 4–1.6	1.3–1.6	1.3–1.5	1 4–1.8		1.3–1.8		
	$\bar{x}$	1 1	1 1	1 4	1.3	1.5	1.5	1 4	1.6		1 6		1 7
	n_1	5	5	5	3	7	6	4	3		4		1
	n_2	5	5	3	3	5	5	3	2		4		
Mg mEq/100 g FFS	range	9 1–10 1	8 2–10.6	7 7–9 7	8 0–10.3	7.9–10.5	7.5–9.8	7.0–10 4			7 1–9.8		
	$\bar{x}$	9.6	9 6	9.0	9.3	9.5	9.2	8.9	8.9		9.2		9.3
	n_1	7	9	8	10	12	11	7	3		7		1
	n_2	7	9	5	7	7	9	5	2		7		

Co tr = ontrol foetuses Gluc = foetuses of glucose loaded mothers $\bar{x}$ = mean value n_1 = number of foetuses n_2 = number of litters

K determined by neutron activation analysis

When during one period determinations from several foetuses from the same litter had been made ($n_1 > n_2$) a mean value for the litt was calculated. Thus within every cell in the table there are n_2 observations, which can be regarded as independent. The mean and the ran of the group were calculated from these n_2 values

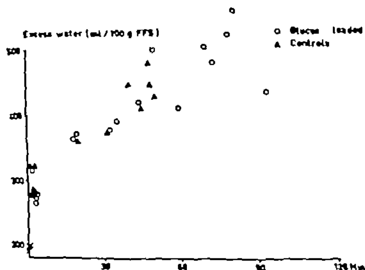


Fig 11 Excess water in heart muscle during anoxia.

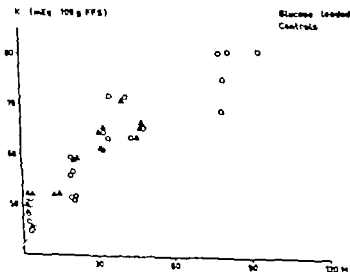


Fig 12 Potassium in heart muscle during anoxia.

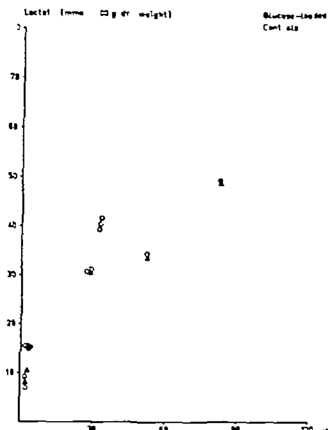


Fig. 13 Lactate in heart muscle during anoxia.

vestigated concurrently In the glucose-loaded group—whose heart activity had a longer duration—the rise in K was much greater at the end of the period of anoxia than that in the control group (an increase of 32 mEq and 18 mEq/100 g FFS respectively) The increase was highly significant in both groups $P<0\ 001$) The mean value for the last animal of each litter was significantly higher in the experimental group than in the control group ($79.9 \pm 1\ 6$ and $67\ 8 \pm 2\ 0$ mean $\pm$ S E. respectively $P<0\ 01$)

Lactate (Fig 13) The concentration rose markedly during the whole period of anoxia No significant difference was present when foetuses from both groups were compared at the same time interval At the end of anoxia the increase was greater in the experimental group than in the control group ($49\ 3 \pm 3.3$ and

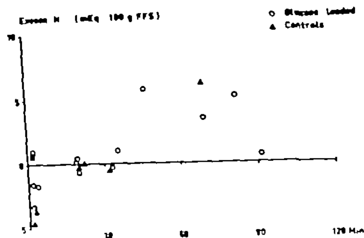


Fig 14 Excess sodium in heart muscle during anoxia

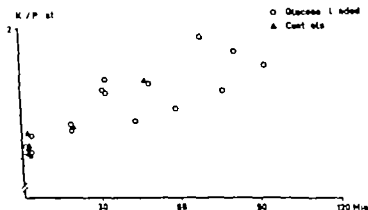


Fig 15 K /P ratio in heart muscle during anoxia

33 4 ± 5 4 mean ± S.E. respectively) In the glucose loaded group the mean value at the end of anoxia for the last animal of each litter was almost significantly higher in the experimental group than in the control group (62.3 and 47.8 respectively $P < 0.05$)

Na and *Na* (Fig 14) The Na content did not change significantly whereas excess Na increased significantly in both groups ($P < 0.01$) A tendency to a decrease in Na did, how

ever occur at the end of the period of anoxia (after about 50 minutes) in the glucose-loaded foetuses

Phosphorus No significant change occurred during anoxia in either group

K /P ratio (Fig 15) The K /P ratio rose considerably in both groups At the end of the period the rise was much greater in the glucose-loaded group than in the untreated one The difference is significant ($P < 0.001$)

Mg The content did not change significantly during the period of anoxia No difference was present between the groups.

Discussion

During anoxia a considerable increase took place in the plasma concentration of potassium, lactate and phosphate in both the glucose-loaded foetuses and the controls while the blood pH fell. On the other hand no definite changes occurred in the calcium, sodium, chloride and protein concentrations. Similar results with respect to potassium, lactate and pH in foetuses under anoxia have been reported previously (*Colldahl* 1947 *Salmi* 1954 *Brandt et al* 1958 *Thalme* 1967)

The rise in potassium concentration of the plasma during the first 50 minutes of anoxia was significantly lower in the experimental group

In the present series the total CO_2 was also determined It decreased during anoxia concurrently with a rise in lactate and a fall in pH.

In the heart muscle a pronounced increase was noted in the total water total potassium and lactate in relation to dry weight. The total quantity of chloride sodium and phosphorus had not undergone any definite change nor had the chloride space *i e* the distribution volume of chloride On the other hand excess sodium—*i e* sodium outside the chloride space—which was initially negative in most foetuses rose during the period of anoxia

If the pronounced increase in total water had to a great extent consisted of an increase in extracellular fluid both the chloride space and the sodium space should have shown a considerable percentage increase Since this did not occur our results indicate

that the increase in water consisted mainly of an increase in intra cellular water This is in accordance with findings in skeletal muscle of working rats in hypoxia (Rooth 1966) The increase took place chiefly during the first 40 minutes. The lower heart water content in the experimental group as compared to the control group at the beginning of anoxia may have been due to the initial hyperglycaemia (*Gelli et al.* 1968) which has an osmotic effect on the cells.

The potassium content of the heart muscle of anoxic foetuses is of great interest. In relation to the dry fat free weight, the potassium content increased by no less than about 30 per cent in the untreated group and by about 60 per cent in the glucose-loaded one This difference was significant. The course of the potassium increase was the same in both series. However higher values were reached by the glucose loaded foetuses which seems to be related to their longer heart activity It was previously shown that the heart muscle had a higher initial glycogen content in the foetuses of glucose-loaded mothers, and that their heart activity had a longer duration under anoxia (*Gelli et al.* 1966 and 1968)

From the point of view of electroneutrality it is inconceivable that potassium can be accumulated to the degree which occurred in our experiments without a concurrent increase in the content of intracellular anions.

In anaerobic metabolism, lactic acid is formed, *i.e.* lactate ions + hydrogen ions. Our results demonstrate that a considerable amount of lactate accumulates in the heart, and that the increase in lactate is greater than the increase in potassium. The possibility exists both that hydrogen ions are buffered intracellularly and that they leave the heart and are buffered in extracellular fluid or in other organs.

Eckel Botschner and *Wood* (1959) attempted to determine the buffering capacity of skeletal muscle by titration of muscle homogenates at 0 C. On titration in the pH interval 6–7 they found a buffering capacity of about 16 mEq/l H per pH unit per 100 g FFS It is possible that the buffering capacity of foetal heart muscle is much greater since its phosphate content is far higher than that of the skeletal muscle of adult animals.

On the other hand, a *considerable amount of hydrogen ions* must necessarily have left the heart muscle cells to permit the great K uptake to occur The falling pH and total CO_2 of the extracellular fluid suggest that hydrogen ions leave the cells. The quantitative role played by the heart in this respect cannot, how ever be established even if it can be envisaged to be considerable since the heart is the only working organ In adult man the potas sium/phosphorus ratio in skeletal muscle is relatively constant, and cannot be increased to any appreciable degree despite the presence of hyperkalaemia (*Bergström* 1962) The marked rise in the potassium/phosphorus ratio above the initial value in the anoxic heart shows that the conditions are not similar to those in the skeletal muscle This confirms that extra anion must have been added to permit the accumulation of potassium

As previously mentioned excess sodium rose during the period of anoxia This can be interpreted either by the occurrence of an outflow of chloride from the cells without a concomitant release of sodium, or by sodium entering the cells or possibly by both events taking place concurrently

Whatever the events may be this nevertheless implies that the intracellular cation-anion balance is changed in such a way that *sodium as well as an extra cation can correspond to a given* amount of intracellular lactate ions

It is known from studies in experimental animals and in man that a reciprocal relation is generally present between intracellular sodium and potassium. Such a reciprocal relation does not, how ever seem to exist in the anoxic foetal heart. Instead both pota sium and excess sodium increase during anoxia

The amount of potassium is well correlated to the total water content (Fig 16) This indicates that the intracellular increase in water in the heart is caused by an increase in the number of osmotically active particles *i.e* on an accumulation of potassium and lactate water is drawn into the cardiac cells which swell This also implies that—although the quantity of potassium per *FFS increases—the intracellular potassium concentration does* not increase or only inappreciably Since the extracellular potassium concentration increases greatly this denotes that the K_i /K_e ratio decreases If potassium is distributed according to the Nernst

equation (*Conway* 1957) this would imply that the resting membrane potential decreases continuously in the heart during anoxia.

One of the basic aims of this study was to ascertain whether the prolongation of heart activity in the foetuses of glucose loaded and of untreated mothers could in fact, be explained by some other factor than the increased glycogen content of the heart in the former group e.g differences with respect to the electrolyte metabolism When foetuses from the respective groups were compared with regard to electrolyte content of muscle after an equally long period of anoxia there was however no definite difference between the groups with respect to any electrolyte. The foetuses of glucose-loaded mothers—which generally had a longer recordable heart activity—consequently continued to accumulate potassium. Their final values were thus significantly higher at the end of anoxia than those in the control foetuses

We have interpreted this to mean that the potassium accumulation as such is not a limiting factor but that it is secondary to the accumulated amount of lactate which, in turn is dependent on the initial amount of glycogen This was greater in the glucose-loaded group (*Gelli et al* 1966 and 1968) which also had the longest recordable heart activity and, therefore also the greatest glycogen utilization

The mechanism of the ion and water accumulation in the anoxic foetal heart is unknown It is nevertheless evident that an active transport is required for this purpose either of potassium or hydrogen ions or through a coupled ion-transport mechanism The energy for this transport is obtained through glycolysis, since the animal is anoxic Sodium presumably increases intracellularly This increase may depend either on an increased sodium permeability i e a passive entry of sodium into the cell, or on a decreased active transport of sodium from the cell Rooth (1966) has recently postulated an active cation pump in skeletal muscle which in hypoxia will pump H out from the cells with a concurrent decrease in Na elimination. The ability to extrude H from the anoxic cells may be one of the vital functions which cease when the energy supply is insufficient because of glycogen depletion

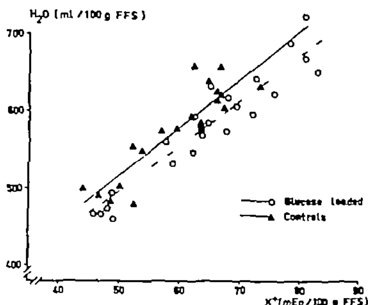


Fig. 16 Potassium correlated to the total water content in heart muscle.

SUMMARY

The electrolyte conditions in the heart muscle and in plasma were studied during anoxia in glucose-loaded and in control foetuses. The changes in plasma electrolytes showed the pattern already known i.e a great increase in lactate and a fall in pH and total CO_2 Initially the glucose-loaded group had lower plasma potassium values than the control group

The water content, potassium and lactate in the heart muscle increased markedly during anoxia The glucose-loaded group reached higher final values of K and lactate Chloride total sodium, phosphorus and magnesium showed no significant changes whereas excess Na increased significantly in both groups. The results indicate that during anoxia an accumulation of lactate takes place intracellularly in the heart muscle and that K and to some extent Na enter the cells with a concurrent release of H from them Water is drawn into the cells through the osmotic effect exerted by the accumulated ions

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EFFECT OF GLUCOSE INFUSION IN THE MOTHER BEFORE DELIVERY ON THE ECG OF RABBIT FOETUSES UNDER ANOXIA

BY

M G GELLI AND F GYULAI

In a previous study the effect was investigated of glucose infusion in the mother before delivery on the heart activity of rabbit foetuses under anoxia (*Gelli et al* 1966 and 1968 a) It was found that asystole appeared much later in the glucose-treated group than in the controls An account is given in this paper of the way in which the various ECG parameters underwent changes during anoxia in both groups

Material and Methods

Twenty six rabbit foetuses removed by laparotomy on the 29th day of pregnancy were investigated. 13 were used as controls and the same number as experimental animals whose mother had been given a glucose infusion before delivery For the technique reference is made to earlier reports (*Gelli et al* 1966 and 1968 a)

The standard ECG in lead I was recorded with an Elema Mingograph 12 B from 5 6 minutes after the onset of anoxia and during the whole experiment, i.e generally as long as any heart activity was present. In some cases however the recording was discontinued after the cessation of ventricular activity The paper speed was 25 mm/second during continuous recording but interesting sections were recorded at a rate of 100 mm/second.

The ECG was analyzed at a magnification of 7 X and with an accuracy of ±0 1 mm, which corresponds to 0 001 second (at the

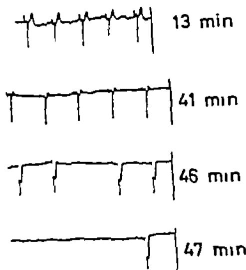


Fig. 1 Control.
13 min Normal ECG *41 min* Slight S–T depression. *46 min* Extreme S–T depression *47 min* Complete A V block ventricular rate < 10/min.

last-mentioned paper speed) Measurements were made routinely every 8–20 minutes, as well as immediately before any major changes (*e g* appearance of S–T depression or A–V block)

Results

Controls (Fig 1 Tables I and II)

Initially there was sinus rhythm, with a mean rate of 85/min. The P waves were positive and the mean P–Q time 60 msec with a relatively small range (40–70 msec) The S–T segment was iso-electric in all but two cases in which it was depressed. The T waves were positive The Q–T time was a mean 185 msec

Table I ECG *Parameters after Varying Periods of Anoxia.*
Contr = Controls. Gluc. = *Glucose loaded Foetuses*

Anoxia (min)	6–10		30–40		41–62		63–87		88–	
Foetuses	Contr	Gluc.	Contr	Gluc.	Contr	Gluc.	Contr (64–69 min)	Gluc. (70–84 min)	Contr	Gluc.
P–Q (msec)										
mean	58	69	73	73	102	136	177	250		
range	40–70	40–160	60–100	50–120	80–140	45–200	175–180	150–360		
No	13	13	12	13	9	11	2	4		
Heart rate (beats/min)										
mean	85	70	68	61	53	36	33	25		
range	60–120	40–93	10–108	19–90	10–115	10–52	20–46	15–52		
Q–T (msec)							(63–74 min)	(65–84 min)		(101–102 min)
mean	185	192	153	180	144	163	146	180		200
range	150–240	160–220	130–200	140–280	100–170	120–200	120–160	120–260		160–240
No	13	13	12	13	9	11	2	7		2
Heart rate (beats/min)										
mean	85	70	68	55	53	33	33	ca 21		
range	60–120	40–93	10–108	19–90	10–115	10–52	20–46	< 10–50		< 10

During the first 40 minutes, the ECG showed comparatively few changes. The rate fell to about 68/min the conduction time increased, and the Q–T time decreased slightly At the end of this period, depression of the S–T segment appeared, and increased markedly during the next few minutes after on an average 49 minutes anoxia, the T waves became negative.

After 52 minutes anoxia, an A–V block appeared with an extremely low ventricular rate (usually ≤10/min) but no concurrent change in atrial rate. From this time onwards, bursts of ventricular extrasystoles frequently occurred.

The conduction time first increased slowly then successively more and shortly before the appearance of A–V block was a mean 100 msec. The heart rate had, however fallen concurrently to slightly over 50/min. The Q–T time, on the other hand, fell continuously despite a coincident decrease in heart rate, and during the period 41–62 minutes anoxia was considerably lower than the initial value i.e around 140 msec.

The mean value of the duration of heart activity in this group was 64 minutes.

Experimental animals (Fig. 2, Tables I and II)

At the beginning of the recording, there was sinus rhythm the mean rate being 70/min. The P waves were positive and the P–Q time about 70 msec. The S–T segment was isoelectric, except in one case in which it was depressed. The T waves were positive The Q–T time was about 190 msec.

During the next 40 minutes the heart rate fell to a mean 61/min whereas the P–Q time and Q–T time were largely unchanged. The conduction time showed a marked, successive increase and in 4 cases in which it could be measured in the period 63–84 minutes anoxia it was a mean 250 msec. Concurrently the heart rate was about 25/min. In the foetuses of the glucose-loaded mothers no major changes occurred in the Q–T time during anoxia Since there was a concomitant marked decrease in the heart rate this must imply a relative shortening of the Q–T time In these foetuses, the mean total duration of heart activity was 82 minutes

After the first 40 minutes these 13 ECG's could be divided into

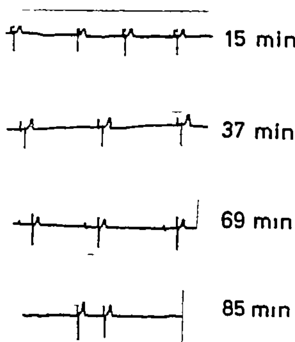


Fig 2. Experimental foetus
15 min Normal ECG *37 min*: Normal ECG S–T segment isoelectric. T wave positive *69 min* Prolonged P–Q time. Mainly unchanged ECG *85 min* Complete A–V block. Ventricular rate ~ 10/min. No S–T depression. T wave positive.

two groups. In 7 cases an S–T depression appeared after about 43 minutes anoxia it subsequently increased and the T waves became negative after a few minutes *i e* at about the same time as in the controls. In this group the heart activity persisted for a mean 71 minutes. In the other 6 cases no S–T depression occurred The T waves remained positive for practically the whole time (87 5 min) The heart activity ceased after a mean 94 minutes anoxia.

Comments

Infusion of glucose in the mother animal before delivery resulted in higher glycogen content of the foetal heart muscle of about

Table II. *Time of Appearance During Anoxia of S–T and T Changes as well of Atrio-Ventricular Dissociation and Total Duration of Ventricular Activity Respectively*

Contr Controls. Gluc.=Foetuses of Glucose-loaded Mothers

Foetuses	Contr	Gluc.
Negative T waves (min anoxia)		
mean	49	67
range	19–74	29–104
S–T depression (min anoxia)		
mean	39	43*
range	10–68	10–75
A V block (min anoxia)		
mean	52	64
range	27 79	29–97
Ventricular activity (min anoxia)		
mean	64	82
range	45–87	54–118

7 cases (no S–T depression in 6 cases)
10 cases (no A V block in 3 cases)

40 per cent. During anoxia this difference from the controls could be demonstrated during the whole period of heart activity In the foetuses of glucose-loaded mothers, the heart activity had a longer duration, but the muscle glycogen—after a continuous decrease in both groups—reached a level of about 0 4 g/100 g wet weight when the heart activity ceased (Gelli et al 1968 a)

Although the ultrastructure of the cardiac muscle cells showed the same pathological changes in both groups, they appeared later in the glucose-loaded animals than in the controls (Gelli et al 1968 b)

Initially the electrolyte values appeared similar and the intra cellular potassium increased with time at the same rate In the final phase this resulted in hig er potassium values in the foetuses of glucose-treated mothers since their heart activity continued for

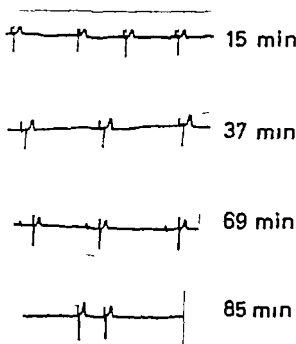


Fig 2 Experimental foetus.
15 min Normal ECG *37 min* Normal ECG S–T segment isoelectric. T wave positive *69 min*: Prolonged P–Q time Mainly unchanged ECG *85 min* Complete A-V block Ventricular rate ~10/min. No S–T depression. T wave positive

two groups In 7 cases an S–T depression appeared after about 43 minutes anoxia it subsequently increased, and the T waves became negative after a few minutes *i.e* at about the same time as in the controls In this group the heart activity persisted for a mean 71 minutes. In the other 6 cases no S–T depression occurred. The T waves remained positive for practically the whole time (87.5 min) The heart activity ceased after a mean 94 minutes anoxia

Comments

Infusion of glucose in the mother animal before delivery resulted in higher glycogen content of the foetal heart muscle of about

Table II. Time of Appearance During Anoxia of S–T and T Changes as well as of Atrio-Ventricular Dissociation and Total Duration of Ventricular Activity Respectively

Contr = Controls. Gluc. = Foetuses of Glucose-loaded Mothers.

Foetuses	Contr	Gluc.
Negative T waves (min anoxia)		
mean	49	67
range	19–74	29–104
S–T depression (min anoxia)		
mean	39	43*
range	10–68	10–75
A–V block (min anoxia)		
mean**	52	64
range	27–79	29–97
Ventricular activity (min anoxia)		
mean	64	82
range	45–87	54–118

7 cases (no S–T depression in 6 cases).
10 cases (no A–V block in 3 cases).

40 per cent. During anoxia this difference from the controls could be demonstrated during the whole period of heart activity. In the foetuses of glucose-loaded mothers the heart activity had a longer duration, but the muscle glycogen—after a continuous decrease in both groups—reached a level of about 0.4 g/100 g wet weight when the heart activity ceased (*Gelli et al.* 1968 a).

Although the ultrastructure of the cardiac muscle cells showed the same pathological changes in both groups, they appeared later in the glucose-loaded animals than in the controls (*Gelli et al.* 1968 b).

Initially the electrolyte values appeared similar and the intracellular potassium increased with time at the same rate. In the final phase this resulted in higher potassium values in the foetuses of glucose-treated mothers, since their heart activity continued for

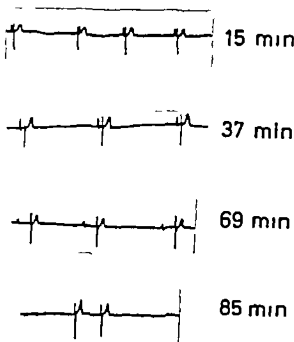


Fig. 2. Experimental foetus.
15 min. Normal ECG. *37 min*: Normal ECG. S–T segment isoelectric. T wave positive. *69 min*. Prolonged P–Q time. Mainly unchanged ECG. *85 min*. Complete A–V block. Ventricular rate ~10/min. No S–T depression. T wave positive.

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Comments

Infusion of glucose in the mother animal before delivery resulted in higher glycogen content of the foetal heart muscle of about

during the later part of anoxia (*Gelli et al.* 1968 c) may also contribute to higher and, therefore more distinct T waves in the latter cases. The differences in P–Q and Q–T times were, however consistently small, and they will be studied in greater detail

SUMMARY

It has previously been shown that rabbit foetuses whose mother had received a glucose infusion before delivery had a longer duration of heart activity under anoxia than the controls. The present study comprises an analysis of the ECG s during the whole period of anoxia in both groups. The mean duration of heart activity was 64 minutes in the controls, and 82 minutes in the glucose-loaded foetuses.

Depression of the S–T segment occurred soon after 40 minutes anoxia in the controls. In 50 per cent of the glucose-loaded foetuses no S–T depression occurred, and in the other 50 per cent it appeared 13 minutes later than in the controls.

The conditions were approximately the same with respect to the T waves. An A–V block appeared in the controls after about 52 minutes anoxia. No block occurred in 3 of the foetuses of glucose-loaded mothers, and in the rest it appeared 12 minutes later than in the controls.

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a longer time The extracellular pH behaved analogously and therefore reached lower values in this group (*Gelli et al* 1968 c)

During anoxia pathological changes in the ECG took place successively but not at the same rate nor to the same extent in the glucose loaded foetuses as in the controls

The most striking difference was with respect to the S–T segment. Depression of this segment is known to be a sign of myocardial ischaemia which may be due either to generalized hypoxia or to a localized disturbance in the oxygen supply to the myocardial cells

The total duration of heart activity in the 6 glucose-loaded cases with no S–T depression was a mean 94 minutes (range 72–118 min) as compared to a mean 71 minutes (range 49–99 min) in the remaining 7 foetuses of glucose-treated mothers. The corresponding duration in the controls was 64 minutes (range 45–87 min) Moreover in the aforementioned 6 cases the T wave was positive for practically the whole time (87.5 min) whereas in the other 7 cases it became negative after about 48 minutes anoxia. Thus normal S–T segments and T waves—reflecting better myocardial function during heart activity under anoxia—were demonstrated in about half the foetuses of glucose-loaded mothers. More detailed ECG studies with several leads are required to establish the cause of the differences observed.

An A V block with low ventricular rate is an expression of poor myocardial function In all controls an A–V block appeared after a mean 52 minutes anoxia whereas no block occurred in 3 experimental animals and in the rest of them the onset was later i.e after about 64 minutes.

The conduction time (P–Q time) increased during anoxia in both groups first slowly and then increasingly rapidly The fact that the foetuses of glucose-loaded mothers finally reached higher values can be explained by the longer duration of their heart activity In addition their slightly lower heart rate may have been a contributory factor The somewhat longer Q–T time in the glucose-loaded foetuses may have been due to the depression of the S–T segment making the T waves isoelectri or negative to a greater extent in the controls than in the foetuses of glucose-treated mothers. The high potassium content of the heart muscle

during the later part of anoxia (Gelli et al 1968 c) may also contribute to higher and, therefore more distinct T waves in the latter cases. The differences in P–Q and Q–T times were however consistently small, and they will be studied in greater detail.

SUMMARY

It has previously been shown that rabbit foetuses whose mother had received a glucose infusion before delivery had a longer duration of heart activity under anoxia than the controls The present study comprises an analysis of the ECG's during the whole period of anoxia in both groups. The mean duration of heart activity was 64 minutes in the controls, and 82 minutes in the glucose-loaded foetuses

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SPERM AGGLUTININS IN MALE BLOOD DONORS

BY

BO FJÄLLBRANT

Introduction

Investigations of sperm antibodies in human males have been performed as studies of a few cases (*Wilson* 1954 1956) as determinations of the incidence in men with azoospermia (*Rümke* 1954) and obstructed vas deferens (*Phadke* and *Padukone* 1964) in a urological clientele (*Bandhauer* 1966) in sterile clienteles (*Fjällbrant* 1965 *Schwimmer et al* 1967) and as comparisons between the incidence in sterile and fertile groups (*Rumke* and *Hellinga* 1959 *Fjällbrant* 1968) Bandhauer included in his investigation a control group of 50 patients who were treated for diseases unrelated to the genital tract in this control group he found no case of sperm agglutinin positivity (defined as a titre ≥ 1 32 with the method of Kibrick)

Knowledge of the presence of sperm antibodies in healthy men is lacking but would be useful for interpreting results of studies in groups selected with regard to fertility or certain diseases The aim of the present investigation was to determine the presence and titre of sperm agglutinins in a group of obviously healthy men not selected with regard to fertility and to compare the results with the incidence in fertile and sterile groups reported in an earlier investigation performed with the same technique (*Fjällbrant* 1968) Although no influence of the ABO blood groups on the results of the earlier investigation was found their possible influence was reconsidered.

Material and Methods

The blood donors were 500 healthy men 20–45 years old (mean 30 years) Blood samples for testing with regard to sperm ag glutinins were taken simultaneous to donation.

The ABO blood group was determined by testing their erythrocytes for antigens and their sera for antibodies.

The blood samples were centrifuged and the decanted sera kept at -30 C until examination.

Spermatozoa. Fresh ejaculates from one donor were used throughout the investigation. He belonged to blood group B and was a secretor of B-substance in his seminal plasma. Before use each ejaculate was controlled for volume, sperm density and percentage of motile spermatozoa. The means of these values for 11 ejaculates used in the sperm agglutinin determinations were as follows volume, 5.5 ml (standard deviation, 0.21 ml) sperm density 105 million per ml (standard deviation, 14 7 million/ml) motile spermatozoa, 55 per cent (standard deviation, 5 7 per cent) The sperm motility was very rapid, and in other respects the samples were also normal.

The test for sperm agglutinins was performed with the method of Kibrick et al (1952) slightly modified

The serum was inactivated by heating at 56° C for 30 minutes and diluted 1 4 with Baker s solution (buffered glucose) A fresh ejaculate was diluted with Baker s solution to a sperm density of 40 million per ml. The diluted semen was mixed with an equal olume of 10 per cent gelatin in Baker's solution. An aliquot of 0 3 ml of the semen-gelatin mixture was mixed with 0.3 ml of the diluted serum, transferred to a small precipitation tube (5×65 mm) and incubated at +37 C for 2 hours. The presence of ag glutinates was assessed macroscopically immediately after incuba tion.—For the titre determination the sperm agglutinin positive sera were serially diluted two-fold with Baker s solution. Each dilution was blended with the semen-gelatin mixture and incubat ed as described above The highest dilution of serum before the blending with the semen-gelatin mixture that gave an obvious agglutination after incubation, was registered as the agglutinin titre

The statistical analysis which involved comparisons between

Table I *Distribution of the Sera According to Sperm Agglutinin Titre*

Negative Sera	Sperm Agglutinin Titre (reciprocal)										Total
	4	8	16	32	64	128	256	512	1024	2048	
476	2	6	5	5	2	2	1	–	–	1	500

Table II *Distribution of Sperm Agglutinin Negative Sera and Sera with Low (≤1 32) and High (≥1 64) Titres*

	Total	Negative Sera	Positive Sera		
			Low Titres	High Titres	Total
Number	500	476	18	6	24
Per cent	100	95.2	3.6	1.2	4 8

Table III *The Distribution of the ABO Blood Groups in the Sperm Agglutinin Positive and Negative Groups. Per Cent Figures in ( )*

	A	O	B	AB	Total
Negative	213	188	53	22	476
	(44.8)	(39.5)	(11 1)	(4 6)	(100 0)
Positive	11	11	2	–	24
	(45.8)	(45.8)	(8.4)	(0 0)	(100.0)
Total	224	199	55	22	500
	(44 8)	(39 8)	(11 0)	(4 4)	(100 0)

distributions was performed with χ^2 tests A difference was called significant when $p < 0\ 05$ highly significant when $p < 0\ 01$ and almost significant when $p < 0\ 1$

Results

The distribution according to sperm agglutinin titre of the sera is given in Table I There were 24 positive sera out of 500 an incidence of 4 8 per cent The sera were classified into low (≤1 32) and high (≥1 64) titre categories There were 18 (3 6

per cent) with low titres and 6 (1.2 per cent) with high titres, see Table II.

The distribution of the ABO blood groups in the sperm agglutinin positive and negative men is given in Table III. Statistical analysis showed that there was no significant difference between the sperm agglutinin positive and negative groups in regard to the ABO blood group distribution ($X^2 = 1.50$ d.f. $= 3$ $p > 0.1$).

Discussion and Conclusions

The test for sperm agglutinins used in this investigation was discussed in detail in an earlier paper (*Fjällbrant* 1968). In the investigation reported in that paper the ABO blood group and the presence and titre of sperm agglutinins was determined in 900 men, of which 400 lived in sterile marriages and 500 in fertile marriages. The ABO blood group distribution was almost the same in the sperm agglutinin positive and negative groups. Also in the present investigation it is noted that the ABO blood group distribution was very similar in the sperm agglutinin positive and negative groups of blood donors. Most notable is that two sperm agglutinin positive males belonged to the B group, although the semen used as antigen came from a man belonging to the B group. The similar blood group distribution in the sperm agglutinin positive and negative groups and the finding of men with sperm antibodies belonging to the B group would not have occurred if the ABO blood group agglutinins were a primary cause of agglutination in the sperm agglutination method used. It can be concluded that the ABO blood factors do not influence the results obtained with Kibrick's gelatin agglutination test.

In the aforementioned investigation the mean age of the men in the sterile group was 32 years (range 21–56 years) and in the fertile group 30 years (range 19–51 years) to be compared to a mean age of 30 years (range 20–45 years) of the blood donors. The present investigation was performed with the same technique and with use of the same semen donor. Since the groups were similar in regard to age and the investigation methods the same comparisons of the results of the investigations may be considered valid.

Table IV *Distribution (Per Cent) of Sperm Agglutinin Negative Sera and Sera with Low (≤1 32) and High (≥1 64) Titres in the Sterile Fertile and Blood Donor Groups Absolute Numbers in ( )*

	Total	Negative Sera	Positive Sera		
			Low Titres	High Titres	Total
Sterile	100.0(400)	93.2	2.5	4.3	6.8
Fertile	100 0(500)	97 4	1 8	0.8	2.6
Blood donors	100.0(500)	95 2	3.6	1.2	4.8

The incidence of sperm agglutinin positivity was 6 8 per cent in the sterile and 2 6 per cent in the fertile group The incidence in the blood donors was 4 8 per cent or midway between the figures for the sterile and fertile groups

In the earlier investigation it was found that the difference with regard to titre level between the sterile and the fertile groups was greatest if a limit was drawn between the titres 1 32 and 1 64 Titres below this limit were called low and titres above high. The distribution of low and high titres in the sterile fertile and blood donor groups is given in Table IV The sterile group had the greatest incidence of high titres The fertile group showed the smallest incidence both of low titres and of high titres The blood donor group showed the greatest incidence of low titres the incidence of high titres in the blood donor group was somewhat greater than in the fertile group but considerably smaller than that of the sterile group

Statistical analysis showed that the differences between the sterile and fertile groups were significant except in regard to low titres A statistical analysis of the difference between the blood donors on one hand and the sterile and fertile groups on the other gave the following results The difference between the blood donors and the fertile group with regard to incidence of positive sera was almost significant ($X^2 = 3\ 40$ d.f $= 1$ $p < 0\ 1$) with regard to low titres also almost significant ($X^2 = 3\ 08$ d.f $= 1$ $p < 0\ 1$) and with regard to high titres not significant ($X^2 = 0\ 40$ d.f $= 1$ $p > 0\ 1$) The difference between the blood donors and the sterile group with regard to incidence of positive sera was not

significant ($X^2 = 1.59$ d.f. = 1 p > 0.1) nor was the difference with regard to low titres ($X^2 = 0.89$ d.f. = 1 p > 0.1). The difference between the blood donors and the sterile group with regard to high titres was however highly significant ($X^2 = 8.30$ d.f. = 1 p < 0.01).

The earlier investigation showed that the sterile and fertile groups differed statistically significantly with regard both to the incidence of sperm agglutinin positive sera and to the incidence of high titres. The blood donor group reported on in this paper showed an incidence of sperm agglutinin positive sera that was just between those of the sterile and fertile groups. With regard to the incidence of high titres the blood donor group was more like the fertile group as the difference between the incidences of high titres of the blood donor group and the fertile group was small and statistically not significant while the difference with regard to high titres between the blood donor group and the sterile group was great and statistically highly significant.

Higher incidence of sperm agglutinin positive sera and sera with high titres was found in the sterile group in comparison to the incidence in the fertile group and the group not selected with regard to fertility. With regard to the incidence of low titres there were no statistically significant differences between the groups. The result of the investigation shows that there was an accumulation of men with sperm agglutinins especially of men with high titres, in the sterile group. This observation indicates that sperm antibodies in men, particularly at high levels, may interfere with fertility.

SUMMARY

The sera of 500 male blood donors, 20–45 years old, were investigated for sperm agglutinins with Kibrick's gelatin agglutination test. No influence of the ABO blood factors in the differentiation of sperm agglutinin positive and negative groups was demonstrated. The incidence of positive sera was 4.8 per cent, 3.6 per cent with low titres (≤1:32) and 1.2 per cent with high titres (≥1:64). The incidence of high titres was significantly

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The incidence of sperm agglutinin positivity was 6.8 per cent in the sterile and 2.6 per cent in the fertile group. The incidence in the blood donors was 4 8 per cent, or midway between the figures for the sterile and fertile groups.

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CERVICAL MUCUS PENETRATION BY HUMAN SPERMATOZOA TREATED WITH ANTI-SPERMATOZOAL ANTIBODIES FROM RABBIT AND MAN

BY

BO FJÄLLBRANT

Introduction

Whether sperm antibodies have an anti-fertility effect has mostly been studied in female animals immunized with semen. In some experiments it has been shown that exposure of spermatozoa to sperm antibodies before insemination can prevent fertilization of ova. *Kiddy et al.* (1959) found that treatment of rabbit semen with high concentrations of anti-rabbit semen immune serum prevented fertilization and that the fertilization rate increased as lower antibody concentrations were used. *Menge et al.* (1962) reported that immune sera produced against bull semen and bull spermatozoa had an anti fertility effect when used to treat bull semen prior to insemination of heifers. *Menge* and *Protzman* (1967) showed that immune sera against rabbit testis, epididymal spermatozoa and semen prevented fertilization in rabbits, but immune sera against rabbit seminal plasma did not. The mechanism of the anti fertility effect was discussed but not definitively explained in these reports. In other experiments a decreased metabolic activity of antibody-treated spermatozoa was observed, e.g. by *Matousek* (1964) who found that the fructolysis of bull seminal spermatozoa was reduced by antibodies against seminal and epididymal bull spermatozoa and by *Mittal et al.* (1965) who found that antisera against bull semen reduced the fructose

lower ($p < 0.01$) in the blood donor group than in a group of men in sterile marriages investigated earlier with the same technique and the same semen donor

Acknowledgements

The Blood Bank (Chief Dr L. Ryttinger) Sahlgrenska sjukhuset, supplied the blood specimens and performed the blood group testing

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Table I. Sperm Density, Percentage of Motile Spermatozoa and Sperm Motility of the Ejaculates

Donor	Sperm Density (millions/ml)	Motile Spermatozoa (%)	Sperm Motility
1	120	60	very rapid
1	135	55	very rapid
1	110	55	very rapid
2	114	65	rapid
3	24	35	slow
4	75	85	very rapid
5	92	65	very rapid
6	102	70	apid
7	36	50	rapid

Seminal spermatozoa were washed 10 times with phosphate-buffered isotonic saline 0.1 M, pH 7.4 called buffered saline below. Then the spermatozoa were diluted to a density of 130 millions/ml with buffered saline and subsequently freeze-pressed 5 times in the x-press described by *Edebo* (1960). This preparation was used for the immunization of 2 rabbits.

Spermatocele contents was drawn by puncture from one patient and centrifuged at 1500 × g for 15 min, most of the supernatant was decanted and the rest transferred with the sediment to a smaller tube and recentrifuged at 650 × g for 15 min. The sedimented spermatozoa were washed 3 times with buffered saline diluted to 200 millions/ml and x-pressed 5 times. This preparation was used for immunization of another 2 rabbits.

The described seminal preparations were kept in small portions at −30 °C until used as antigen.

For preparation of rabbit antisera *injections* were given subcutaneously and intravenously. The subcutaneous injections consisted of 0.5 ml seminal plasma, 0.5 ml of the suspension of seminal spermatozoa and 1.0 ml of the suspension of spermatocele spermatozoa, respectively, emulsified in an equal volume of Freund's complete adjuvant (paraffin oil + Arlacel® + mycobacteria). The schedule implied weekly subcutaneous injections for 6 weeks, the last week supplemented with 3 intravenous injec

utilization the oxygen uptake and the lactic acid production of bull spermatozoa

No studies on the fertilization capacity of antibody treated spermatozoa have been performed in man and will probably not be done except as chance ordains. *Wilson* (1954 1956) in studies of three men with sperm antibodies observed that the spermatozoa quickly lost their motility in cervical mucus at postcoital and *in vitro* tests In a more extensive study on men with sperm antibodies *Fjällbrant* (1968 b) found an interrelation between high levels of sperm antibodies in blood reduced penetration of cervical mucus by their spermatozoa and sterility The investigation did not show however that the reduction of the penetration ability and the sterility were actually caused by the sperm antibodies

The aim of the present investigation was to study the mucus penetration ability of human spermatozoa treated with rabbit antisera against spermatozoa and seminal plasma and with sera from men with naturally occurring sperm antibodies

Material

Spermato oa

Semen samples from 7 donors without sperm antibodies were used. All donors were married and had become fathers within the previous 5 years Six of them had 2 or more children Sperm density percentage of motile spermatozoa and sperm motility of the semen samples are given in Table I Three ejaculates from donor 1 were used for experimental series I–III and one ejaculate from each of donors 2–7 for series IV

Rabbit antisera

A pool of normal ejaculates from 40 donors which had been kept at −30 C was centrifuged at 330 ×g for 15 min and the spermatozoa harvested The supernate was recentrifuged at 2000 ×g for 30 min

Seminal plasma decanted after the recentrifugation was used for immunization of 4 rabbits

Table II. *Sperm Agglutinin Titre and Sperm Immobilizing Activity of the Sera Used for Treatment of Spermatozoa*

Serum	Agglutinin Titre	Immobilizing Activity
Rabbit control serum	0	8 h. 15 min.
Anti-seminal plasma serum	1 65,536	12 min.
Anti-seminal spermatozoa serum	1 8 192	15 min.
Anti-spermatocele spermatozoa serum	1 1,024	55 min.
Human male control serum	0	60 h.
Patient 1 (sterile)	1 128	3 h. 15 min.
Patient 2 (sterile)	1 256	4 h.
Patient 3 (sterile)	1 2,048	4 h. 30 min.
Patient 4 (sterile)	1 8	30 h.
Patient 5 (fertile)	1 8	38 h.
Patient 6 (fertile)	1 16	42 h.

measure of immobilizing activity of a serum was the time required to reduce the percentage of motile spermatozoa from about 70 to 10 in the presence of complement. The same semen donor as for the agglutinin titre determinations was used.

Determination of mucus penetration by spermatozoa

The cervical mucus penetration of spermatozoa was determined with the method of *Kremer* (1965) slightly modified. Cervical mucus samples were taken from women at the expected time of ovulation. The penetrability was tested with semen from the donor used for the sperm antibody tests. Selected for use were 10 readily penetrated voluminous samples of mucus one from each of 10 women. The mucus samples were transparent and had a high spinnbarkeit and such a low viscosity that the mucus could be easily drawn up into capillary tubes. A column of cervical mucus, about 40 mm long, was drawn into a capillary tube the inner diameter of which was 0 7 mm. The ends of the tube were sealed with modeling clay The sealed tube was stored at +4 C until used some days later Then one of the seals was removed and the other partly pushed into the capillary tube so that a mucus

tions of the preparation (0 25 0 25 and 0.50 ml, respectively) given every other day One week after the last injection the animals were bled Two rabbits were injected only with adjuvant. Pre-immunization bleedings were taken The sera were investigated for sperm agglutinins sperm immobilizing antibodies and precipitating antibodies. Sera from the pre-immunization bleedings and sera from the rabbits injected with adjuvant only had no agglutinating or precipitating antibodies and showed a low sperm immobilizing activity The antiserum against each preparation which showed the highest agglutinin titre the greatest immobilizing activity and the largest number of lines in double diffusion-in-gel analysis was selected for this study In the mucus penetration studies a pre immunization bleeding was used as a control (rabbit control serum) The sperm agglutinin titres and the sperm immobilizing activities of the used sera are given in Table II

Patient sera

Blood was taken from 4 male patients in the sterility clientele of the clinic who were known to have sperm antibodies in the blood and from 2 fertile men with sperm antibodies. Blood from one fertile man without sperm antibodies was used as a control (human male control serum) After centrifugation of the blood the decanted sera were kept at −30 C until used. The sperm agglutinin titre and the sperm immobilizing activity of the sera were determined. These values are given in Table II

Methods

Determination of sperm agglutinin titre

The sperm agglutinin titre of both the rabbit and human sera was determined with the macroscopic direct sperm agglutination method of *Kibrick et al* (1952) slightly modified as described in an earlier paper (*Fjällbrant* 1968 a) Semen from one donor was used for all the determinations

Determination of sperm immobilizing activity

The sperm immobilizing activity of the sera was determined as described in an earlier paper (*Fjällbrant* 1968 b) The employed

Table III *Cervical Mucus Penetration and Motility in Mucus, Spermatozoa from Donor 1 Exposed to Various Antisera*

Test Mixture		Penetration		Sperm Motility in Mucus
Series I a. Rabbit antisera at sperm-agglutinating concentration				
		Mucus 1	*Mucus 2*	
Semen		≥ 30 mm	≥ 30 mm	very rapid
Semen	rabbit control serum	≥ 30 mm	≥ 30 mm	very rapid
Semen	anti-seminal plasma serum	≤ 5 mm	≤ 5 mm	in loco
Semen	anti-seminal spermatozoa serum	≤ 5 mm	≤ 5 mm	in loco
Semen	anti-spermatocele spermatozoa serum	10 mm	14 mm	in loco
Series I b. Rabbit antisera at non-agglutinating concentration				
		Mucus 3	*Mucus 4*	
Semen		≥ 30 mm	≥ 30 mm	very rapid
Semen	rabbit control serum	≥ 30 mm	≥ 30 mm	very rapid
Semen	anti-seminal plasma serum	≥ 30 mm	≥ 30 mm	in loco
Semen	anti-seminal spermatozoa serum	≥ 30 mm	≥ 30 mm	in loco
Semen	anti-spermatocele spermatozoa serum	≥ 30 mm	≥ 30 mm	in loco
Series II. Human sera with sperm antibodies at high titre				
		Mucus 5	*Mucus 6*	
Semen		≥ 30 mm	≥ 30 mm	very rapid
Semen	human male control serum	≥ 30 mm	≥ 30 mm	very rapid
Semen	patient serum 1	≤ 5 mm	≤ 5 mm	in loco
Semen	patient serum 2	≤ 5 mm	≤ 5 mm	in loco
Semen	patient serum 3	≤ 5 mm	≤ 5 mm	in loco
Series III. Human sera with sperm antibodies at low titre				
		Mucus 7	*Mucus 8*	
Semen		≥ 30 mm	≥ 30 mm	very rapid
Semen	human male control serum	≥ 30 mm	≥ 30 mm	very rapid
Semen	patient serum 4	20 mm	27 mm	in loco
Semen	patient serum 5	20 mm	23 mm	slow
Semen	patient serum 6	≥ 30 mm	≥ 30 mm	rapid

drop the size of a pinhead was formed at the other end. This end was immersed into a chamber containing the semen to be tested. After 3 hours in a moist atmosphere at +37 C the extent of penetration was read microscopically A penetration ≥30 mm was classified as normal a penetration 6–29 mm as moderately reduced and a penetration ≤5 mm as highly reduced.

The motility of the *spermatozoa which had penetrated into the* mucus was registered as very rapid rapid or slow movement for ward or as motility only *in loco* without forward movement.

Experimental

Portions of semen were distributed to small precipitation tubes and mixed with test serum Besides the antisera one rabbit or human control serum was used in each series as well as one untreated semen portion The tubes were incubated at +37 C for 1 hour After incubation a drop of the contents was studied by microscopy to estimate motility and agglutination of the spermatozoa and the rest was transferred to the semen chamber for determination of cervical mucus penetration of the *spermatozoa*

The results are summarized in Tables III and IV In all of the series the untreated spermatozoa and the spermatozoa mixed with the control sera showed normal penetration and the motility in mucus was rapid or very rapid with the exception of one semen sample (donor 3) In series 1–3 the penetration of the spermatozoa of each semen portion treated or untreated was tested with mucus from two different females for each portion the results were similar with the two mucus samples In series 4 each semen portion was tested with one mucus sample

Series I a *Spermato oa from one donor treated with various rabbit antisera at sperm-agglutinating concentration* (Table IV)

To 4 aliquots *of 0 4 ml* semen *from donor 1 was added 0 03 ml* anti seminal plasma serum 0 1 ml anti seminal spermatozoa serum 0 1 ml anti-spermatocele spermatozoa serum and 0 1 ml rabbit control serum respectively These volumes were chosen because the antisera added to the semen in these proportions had

Series II. *Spermatozoa from one donor treated with human sera with sperm antibodies at high titre* (Table III)

In this series 0.2 ml serum from each of 3 patients with high sperm antibody levels was added to aliquots of 0.2 ml semen from donor 1 After incubation there was a moderate agglutination and a clearly reduced sperm motility

The results were uniform as all the exposed spermatozoa had a highly reduced penetration and a motility in mucus only *in loco*

Series III. *Spermatozoa from one donor treated with human sera with sperm antibodies at low titre* (Table III)

In this series 0.2 ml of serum from each of 3 men with low sperm antibody levels was added to 0.2 ml aliquots of semen from donor 1 After incubation there was no agglutination and a very rapid sperm motility

The spermatozoa exposed to the serum which had the lowest immobilizing activity (no. 6) had normal penetration, while the spermatozoa of the other serum mixtures had a moderately reduced penetration The serum from the sterile man (no 4) had the highest immobilizing activity and the spermatozoa treated with this serum had a motility in mucus only *in loco* while the spermatozoa treated with serum from patient 5 had a slow and the spermatozoa treated with serum from patient 6 had rapid movement forward in mucus

Series IV *Spermatozoa from various donors treated with rabbit and human antiserum* (Table IV)

To 0 4 ml semen from donors 2–7 was added 0 03 ml anti-seminal plasma serum, and to 0.2 ml semen from the same donors was added 0 2 ml serum from one of the patients with a high sperm antibody level (no. 1) After incubation all the mixtures showed tail-to-tail agglutination The motility was decreased, but to a lesser degree in those samples where the initial motility was very rapid and the percentage of motile spermatozoa high

With the exception of the semen aliquot from the donor with the highest percentage of motile spermatozoa (no 4) which was

Table IV *Series IV Cervical Mucus Penetration and Motility in Mucus of Spermatozoa from Different Donors Exposed to Rabbit Antiserum Against Seminal Plasma or Serum from a Man with Sperm Antibodies*

Donor	Semen		Semen + Anti-seminal Plasma Serum		Semen + Serum from Patient 1	
	Pene-tration	Motility in Mucus	Pene-tration	Motility in Mucus	Pene-tration	Motility in Mucus
2	≥30 mm	rapid	≤ 5 mm	in loco	≤ 5 mm	in loco
3	≥30 mm	slow	≤ 5 mm	none	≤ 5 mm	none
4	≥30 mm	very rapid	≥30 mm	in loco	23 mm	in loco
5	≥30 mm	very rapid	12 mm	in loco	8 mm	in loco
6	≥30 mm	rapid	≤ 5 mm	in loco	≤ 5 mm	in loco
7	≥30 mm	rapid	≤ 5 mm	in loco	≤ 5 mm	in loco

Comment: Donors 2–5 were tested with mucus no 9 and donors 6 and 7 with mucus no 10

proved to cause a moderate agglutination of the tail to-tail type but to leave most of the spermatozoa free with rather rapid motility after incubation for 1 hour at +37 C.

The spermatozoa exposed to anti-seminal plasma serum and anti-seminal spermatozoa serum showed a highly reduced penetration and the spermatozoa exposed to anti spermatocele spermatozoa serum had a moderately reduced penetration The spermatozoa in all the antiserum mixtures had a motility in mucus only *in loco* without forward movement.

Series I b *Spermatozoa from one donor treated with various rabbit antisera at non-agglutinating concentration* (Table III)

Only one-tenth of the volume of each of the rabbit antisera used in series 1 a was added to the aforementioned volume of semen from donor 1 After incubation there was no sperm agglutination and very rapid sperm motility

The mucus penetration was normal The motility of the spermatozoa in mucus was however reduced to motility only *in loco*

Series II. *Spermatozoa from one donor treated with human sera with sperm antibodies at high titre* (Table III)

In this series 0.2 ml serum from each of 3 patients with high sperm antibody levels was added to aliquots of 0.2 ml semen from donor 1 After incubation there was a moderate agglutination and a clearly reduced sperm motility

The results were uniform as all the exposed spermatozoa had a highly reduced penetration and a motility in mucus only *in loco*

Series III. *Spermatozoa from one donor treated with human sera with sperm antibodies at low titre* (Table III)

In this series 0.2 ml of serum from each of 3 men with low sperm antibody levels was added to 0.2 ml aliquots of semen from donor 1 After incubation there was no agglutination and a very rapid sperm motility

The spermatozoa exposed to the serum which had the lowest immobilizing activity (no. 6) had normal penetration, while the spermatozoa of the other serum mixtures had a moderately reduced penetration. The serum from the sterile man (no 4) had the highest immobilizing activity and the spermatozoa treated with this serum had a motility in mucus only *in loco* while the spermatozoa treated with serum from patient 5 had a slow and the spermatozoa treated with serum from patient 6 had rapid movement forward in mucus.

Series IV *Spermatozoa from various donors treated with rabbit and human antiserum* (Table IV)

To 0 4 ml semen from donors 2–7 was added 0.03 ml anti-seminal plasma serum, and to 0.2 ml semen from the same donors was added 0 2 ml serum from one of the patients with a high sperm antibody level (no 1) After incubation all the mixtures showed tail-to-tail agglutination. The motility was decreased but to a lesser degree in those samples where the initial motility was very rapid and the percentage of motile spermatozoa high

With the exception of the semen aliquot from the donor with the highest percentage of motile spermatozoa (no. 4) which was

treated with antiseminal plasma serum the penetration was reduced in all cases. In four cases (nos 2 3 6 and 7) it was highly reduced when the semen samples were treated with either the rabbit antiserum or the patient serum. With both antisera the motility in mucus was reduced to a motility *in loco* in five cases (nos 2 and 4–7) The spermatozoa from the donor with the lowest percentage of motile spermatozoa and the least motility (no 3) showed no motility in mucus.

Discussion and Conclusions

The effect on penetration of selected cervical mucus by treatment of spermatozoa in normal ejaculates from one donor with different volumes of rabbit antiserum against seminal plasma, seminal spermatozoa and spermatocele spermatozoa and with serum from men with different levels of sperm antibodies was studied. To investigate whether and to what extent spermatozoa from other men were also affected by sperm antibodies semen samples from six other fertile men were treated with rabbit anti seminal plasma serum and with serum from a man with sperm antibodies

The suitability of the methods for the determination of sperm agglutinin titre and sperm immobilizing activity of sera has been discussed in earlier papers (*Fjällbrant* 1968 a and b) as has the applicability of the method for the determination of mucus penetration of spermatozoa (*Fjällbrant* 1968 b) The method used for immunization of the rabbits provided antisera with very high sperm agglutinating and immobilizing activities. Of the rabbit antisera the anti seminal plasma antiserum had the greatest agglutinating and immobilizing activity The anti seminal spermatozoa serum had a lesser activity and least was the activity of the serum against spermatocele spermatozoa. There was a similar trend of differences in precipitin content as noted in the double diffusion-in gel patterns which will be reported on in a separate paper

Because both the untreated spermatozoa and the spermatozoa treated with control sera showed normal penetration and unimpaired motility in mucus it can be assumed that the reduction of penetration and motility observed in spermatozoa treated with antisera was caused by antibodies in the immune sera

With regard to the discussion on the antigenicity of seminal plasma versus spermatozoa it is notable that antisera against seminal plasma seminal spermatozoa as well as spermatocele spermatozoa had immobilizing and agglutinating activity and caused reduction of penetration of the spermatozoa treated. Although the anti-seminal plasma serum contained some antibodies against spermatozoa (demonstrated with diffusion-in-gel technique) its very high agglutinating, immobilizing and penetration-reducing activity must presumably be referred also to its high content of antibodies against secretions from the male adnexal glands. As these penetration-reducing antibodies might be supposed to prevent spermatozoa from fertilizing ova the finding of penetration-reducing antibodies against human seminal plasma is in certain respects opposite to the finding of *Menge* and *Protzman* (1967) that rabbit testicular material and not seminal plasma was the source of antigens that induced antibodies capable of preventing spermatozoa from fertilizing ova in does. From the present investigation it seems evident that human spermatozoa *per se* can elicit production of sperm agglutinating, immobilizing and penetration-reducing antibodies, because the spermatocele spermatozoa used had not been in contact with seminal plasma

Volumes of rabbit antisera were added to the semen aliquots to cause only a moderate agglutination and a moderate reduction of sperm motility Although most of the spermatozoa had a rather rapid motility after incubation the mucus penetration of the treated spermatozoa was highly reduced. When the same volumes of semen were treated with one tenth of these immune serum volumes the spermatozoa seemed to be unaffected after incubation, but still there was an obvious decrease of motility in the cervical mucus although the penetration was normal. The sera from male patients which contained sperm agglutinating and immobilizing antibodies (but no detectable precipitins) had largely the same effect on spermatozoa as the rabbit antisera Sera with high antibody concentrations considerably reduced the penetration and the motility of the spermatozoa in mucus. Sera with low antibody concentrations reduced the penetration and the motility to a lesser degree which was proportional to the immobilizing activity of the sera Thus it seems clear that the reduction of the

penetration of antibody treated spermatozoa depends on the concentration of the antibodies

When semen aliquots from six different fertile donors were treated with equal amounts of rabbit antiserum or patient serum, both containing sperm antibodies the spermatozoa from all of the donors were affected but not to the same extent. The motility in mucus was strongly decreased in all cases, and the penetration was highly reduced in four cases The spermatozoa from the donor who had the highest percentage of motile spermatozoa and very rapid sperm motility had however normal penetration after treatment with the rabbit antiserum and moderately reduced penetration after treatment with patient serum. The penetration of spermatozoa from the other donor who had very rapid sperm motility was moderately reduced by both types of antisera Thus the reduction of mucus penetration by antibody treated spermatozoa appears to depend not only on the antibody concentration but also on intrinsic properties of the spermatozoa

In an earlier investigation (*Fjällbrant* 1968 c) it was found that spermatozoa from men with sperm antibodies showed degrees of motility reduction and agglutination which could be correlated to the sperm antibody level in the blood. The spermatozoa had a reduced mucus penetration which could also be correlated to the sperm antibody level in the blood (*Fjällbrant* 1968 b) From the present investigation it seems clear that normal spermatozoa treated with antibodies behave similarly to spermatozoa from men with sperm antibodies in the blood.

The results of the present investigation show that antibodies against human spermatozoa as well as antibodies against seminal plasma both those produced in rabbits and those naturally occurring in males cause a reduction of the mucus-penetrating ability of spermatozoa The degree of impairment seems to depend on the antibody concentration as well as on the intrinsic properties of the spermatozoa Reduction of the penetration ability of spermatozoa diminishes the possibility of spermatozoa reaching the ovum and fertilizing it Although there are other conceivable ways for sperm antibodies to render spermatozoa incapable of fertilization the reduction of penetration ability of spermatozoa seems to be a likely mechanism for sperm antibodies to cause sterility in men.

SUMMARY

Semen aliquots from one donor were treated with different volumes of rabbit antisera against seminal plasma seminal spermatozoa and spermatocele spermatozoa and with sera from men with different concentrations of sperm antibodies in the blood. The penetration of the antibody-treated spermatozoa in selected cervical mucus was investigated. Low concentrations of sperm antibodies reduced the sperm motility in mucus and high concentrations reduced both the sperm motility in mucus and the extent of sperm penetration. When semen from six fertile donors was treated with rabbit antiserum against seminal plasma and with serum from a man with high sperm antibody concentration the spermatozoa from all the donors were affected, but the penetration of spermatozoa from ejaculates with high percentages of motile spermatozoa and very rapid sperm motility was less reduced. The results of the investigation show that antibodies against human spermatozoa as well as antibodies against seminal plasma both those produced in rabbits and those naturally occurring in males, cause reduction of the cervical mucus-penetrating ability of spermatozoa The degree of impairment depends on the antibody concentration and the intrinsic properties of the spermatozoa. Reduction of mucus penetration ability of spermatozoa seems to be a likely mechanism for sperm antibodies to cause sterility in men.

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TREATMENT OF TRICHOMONAS VAGINITIS WITH NIFURATEL

BY

H. GJØNNÆSS AND J CHR. AURE

Trichomonas vaginalis infections are a daily problem for most gynaecologists. In unselected material from Reims (*Chappaz* and *Bertrand* 1965) trichomonas infections were found in 10 per cent of 1717 pregnant patients a similar incidence was found in Manchester (*Watt* 1965)

Trichomonas infections occur most often among women of childbearing age but also can appear after the menopause in young girls or even in the new-born (*Dagenais-Pérusse et al.* 1964)

There is little doubt that trichomonas vaginalis can be transmitted at coitus. Chappaz and *Bertrand* (1965) refer to various papers in which trichomonas vaginalis infections have been found in the male partner in 0–100 per cent of cases.

The importance of systematic therapy as opposed to simply local treatment in the vagina is now generally accepted. *Chappaz* and *Bertrand* (1965) noted that in 292 women with vaginal infections, trichomonas vaginalis was also present in the urethra in 190 (65 per cent) In 505 Norwegian patients with trichomonas vaginitis the organism was discovered in the urethra in 80.8 per cent and in the cervix in 79 4 per cent (*Ødegaard*, 1962 a)

Experience over many years has confirmed the failure of treatment of vaginal trichomonas infections using local therapy alone The appearance of metronidazole therefore represented an advance as one could now use a preparation for systemic treatment. The effectiveness of metronidazole seems to be proven in

several large series the frequency of successfully treated cases has been shown to be between 80 per cent and 99 per cent (*Watt* and *Jennison* 1962 *Sharman* 1963 *Peterson et al* 1966)

The problem of trichomonas vaginalis developing resistance to therapy has been investigated. *Ødegaard* (1962 b) tried in vain to produce an increased *in vitro* resistance by culturing trichomonas vaginalis in media with low concentrations of metronidazole *De Carneri* (1966) however succeeded with a somewhat similar technique *Jennison et al* (1961) found no increased resistance to metronidazole in trichomonas vaginalis from patients who had undergone one course of therapy *Arnold* (1966) reported a success rate of 92.6 per cent in 140 patients treated in 1960–61 but in 1965–66 using a presumably similar method of treatment in a comparable group of 72 patients he found a cure rate of only 68 per cent *Svendsen* (1962) in a series of 76 patients reported a cure rate of 97 per cent In a newly completed study from the same area the success rate in 120 patients was 73 per cent (*Aure* and *Gjonnaess*) Whereas some authors maintain that the reduced effectiveness must be due to the failure of the active substance in reaching the organisms the results from Switzerland and Norway imply that one should be prepared for possible development of resistance against metronidazole

A new compound has been synthetized by Polichimica Sap Milano which may offer alternative therapy thus avoiding the problem of resistance and thereby improve the cure rate. The substance is a nitrofuran derivative with the generic name nifuratel (Fig 1) Nifuratel is a yellow crystalline substance difficult to dissolve in water or the usual organic solvents insoluble in mineral acids and alkali but soluble in dimethylformamide Extensive investigations have shown that nifuratel is practically non toxic both after short and long term administration (*Scuri* 1966) There were no effects shown on various organic functions on haemopoiesis spermatogenesis or ovulation. There was no teratogenic effect demonstrable in mice rats or rabbits and no tendency to accumulate *In vitro* studies carried out at the University of Pisa (*Imperato* 1963) showed a marked trichomoni

In the Scandinavian countries th preparation will be marketed under the name Polmiror[R]



5-[(methylthio)methyl]-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone

Fig. 1 Nifuratel.

cidal effect (total inhibition of growth with a concentration of 0.25 MCG/cc). *In vivo* trials (intraperitoneal inoculation with trichomonas in mice) showed a good trichomonicidal effect both when nifuratel was administered orally and directly into the peritoneal cavity. It was also shown that nifuratel was effective against various Gram positive and Gram negative bacteria, and also in higher concentrations against various fungi, including 3 strains of Candida.

Several clinical studies have been published and some of these are shown in Table I. Regarding the dosage of nifuratel, the results of *Arnold* (1966) and *Vartiainen* and *Widholm* (1966) imply that it is important to achieve a high vaginal concentration. *Arnold* achieved the best results with either a combined treatment (1 tablet of 0.2 g twice daily + 1 vaginal tablet of 0.25 g twice daily both for 10 days) or with vaginal treatment (1 vaginal tablet twice daily for 20 days) alone. Both these schemes gave a success rate of 85 per cent. *Sarnella* (1966) administered an oral dose of 0.2 g three times daily for 7 days + 1 vaginal tablet daily for 15 days and achieved a success rate of 95 per cent. The conclusion seems to be that combined oral and vaginal treatment yields the best result. Almost as good results can be achieved with simple vaginal treatment, but these patients have a higher recurrence rate.

It was decided to evaluate the effectiveness of nifuratel in a controlled population and observe differences in response to dosage and treatment failures.

Table I. *Nifuratel—Clinical Reports*

Author	Department	Year	Patients	Treatment	Cure Rate
Arnold M	Universitätsfrauenklinik Bern	1966	485	oral vaginal comb	64 % 83–93 % 75–91 %
Leoni R.	Obstetric and Gynecologic Clinic University of Florence	1964	30	comb	93 %
Sagone I	Obstetric and Gynecologic Clinic University of Milan	1965	61 20	oral comb	82 % 70 %
Sarnella, A.	Obstetric and Gynecologic Clinic University of Siena	1966	400	comb.	95 %
Vartiainen E. and Widholm O	Central Hospital University of Helsinki	1966	15 20	oral vaginal	47 % 80 %

All results are primary control examinations 1–3 months later where done in all reports revealed less favourable results

In none of the reports was there mention of side effects except for 1 case with vasomotor reaction after consumption of alcohol.

Material and Methods

The series consists of all patients with trichomonas vaginalis infections seen in the gynaecological out-patient department at Lillehammer from March 1966 to January 1967 In most cases the diagnosis was verified by direct microscopy on fresh preparations stained with 0 25 % Brilliant-Cresyl blue in isotonic saline This solution stains leucocytes and epithelial cells but not trichomonas vaginalis They still exhibit approximately the same degree of motility as in isotonic saline solution

An attempt was made to carry out the treatment continuously without interruptions due to menstruation In two patients this was not successful as menstruation occurred earlier than expected and their course of treatment was temporarily discontinued during menstruation. The other patients were examined at least 10 days after the end of the course of therapy All patients received instructions to return if they suspected a recurrence In no

Table II. *Age Distribution*

Age Years	Number of Patients
16–19	11
20–29	36
30–39	22
40–49	19
50–59	7
Total	95

patient was the follow-up period less than 2 months. Those patients in whom trichomonas could be demonstrated within the first 4 months, even if the primary treatment were succesful were termed not cured

The patients were divided into two groups, both receiving nifuratel. Therapy was given in two courses, I and II The first group (60 patients) received 1 oral tablet (0.2 g) three times daily for 7 days, and the second group (30 patients) 1 oral tablet three times daily for 10 days. Both groups received 1 vaginal tablet (0.25 g) every evening for 10 days. The division into groups was carried out randomly irrespective of clinical factors such as coexistent cervicitis. (The term cervicitis is here meant to describe a condition with muco-purulent discharge possible erosion of the vaginal cervix and subjective discomfort in the form of pressure—tension and pain in the abdomen)

The second course of therapy consisted of 1 oral tablet three times daily for 10 days together with 1 vaginal tablet twice daily for 10 days. In all cases it immediately followed the examination which revealed persistent live trichomonas vaginalis. An attempt was made to give therapy to all sexual partners but for various reasons only 67 were treated, as shown in Table V The partner received the same number of oral tablets as the patient.

χ^2-test was used to determine if there were any statistical difference in the results between the different groups.

Table I *Nifuratel—Clinical Reports*

Author	Department	Year	Patients	Treat ment	Cure R
				oral	64 %
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				comb.	75-91
Leoni R	Obstetric and Gyne cologic Clinic Uni versity of Florence	1964	30	comb	93 %
Sagone I	Obstetric and Gyne-cologic Clinic Uni versity of Milan	1965	61	oral	87 %
			20	comb	70 %
Sarnella A	Obstetric and Gyne-cologic Clinic Uni versity of Siena	1966	400	comb.	95 %
Vartiainen E. and Widholm O	Central Hospital University of Helsinki	1966	15	oral	47 %
			20	vaginal	80 %

All results are primary control examinations 1–3 months later where done in all reports revealed less favourable results.
In none of the reports was there mention of side effects, except for 1 case with vasomotor reaction after consumption of alcohol.

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χ^2-test was used to determine if there were any statistical dif ference in the results between the different groups.

Results

The results of the treatment can be seen in Table III. (In two instances the treatment was discontinued and three patients did not return for examination after the first course) There was no difference in the results between the two groups. The duration of symptoms did not influence the results of treatment, neither did parity nor various hormonal states such as diabetes pregnancy menstrual disorders or menopause.

Of 60 patients, who had vaginitis alone 49 were cured. Of 30 patients, who also had cervicitis 20 were cured (Table IV) This difference is not statistically significant.

In those cases where both sexual partners were treated, the success rate after one course was 73 per cent (49/67) (Table V) If the partner was not treated the success rate was 43 per cent (10/23) This difference is statistically significant ($p<0\,05$) After two courses the success rate was 82 per cent (55/67) when the partner was treated, and 61 per cent (14/23) if he was not treated

The effect of nifuratel in relation to other treatment can be seen in Table VI The earlier treatment in most cases had been begun by other doctors and the unsatisfactory results of this therapy had motivated referral of the patient to hospital. Approximately half of the patients who were treated primarily with nifuratel or with metronidazole without effect responded when the other preparation was used. In the group of patients not cured with other treatment (= local treatment of different forms) the effect of nifuratel was approximately the same as overall. Of the 4 patients who failed to respond to nifuratel 3 had some benefit from metronidazole. Among the patients reported as uncured after 2 courses of nifuratel and 1 course of metronidazole there was one who was cured after a total of 3 courses of nifuratel and one after a total of 4 courses alternating with other forms of local treatment Two patients received metronidazole orally and nifuratel vaginal tablets twice daily for 10 days and both were cured.

No serious side effects were recorded. Seven patients who previously had completely regular menstruation began menstruating 1 week too early Urticaria occurred in 2 cases in one of

Table III. *Results of Treatment with Nifuratel*

Course I				Course II				
Oral Treatment (Days)	N	Cured	Not Cured	N	Cured	Not Cured	Cured After Two Courses	%
7	60	39	21	15	7	8	46	77
10	30	20	10	10	3	6	23	77

One patient was not controlled after course II.

Table IV *Results in Relation to Local Findings*

	Course I			Course II				
	N	Cured	Not Cured	N	Cured	Not Cured	Cured After Two Courses	%
Vaginitis	60	42	18	15	7	7	49	82
Cervicitis and Vaginitis	30	17	13	10	3	7	20	67

One patient was not controlled after course II.

Table V *Results in Relation to Treatment of Sexual Partners*

	Course I			Course II				
	N	Cured	Not Cured	N	Cured	Not Cured	Cured After Two Courses	%
Partners treated	67	49	18	14	6	7	55	82
Partners not treated	23	10	13	11	4	7	14	61

One patient was not controlled after course II.

Results

The results of the treatment can be seen in Table III (In two instances the treatment was discontinued and three patients did not return for examination after the first course) There was no difference in the results between the two groups. The duration of symptoms did not influence the results of treatment neither did parity nor various hormonal states such as diabetes preg nancy menstrual disorders or menopause.

Of 60 patients who had vaginitis alone, 49 were cured. Of 30 patients who also had cervicitis 20 were cured (Table IV) This difference is not statistically significant.

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tions are given in the opposite order It is reasonable to assume that this response to therapy depends either on the local condi tions with poor distribution of the active substances or on resistant organisms. If one accepts that the cervical canal is one of the more difficult areas for the active substances to gain access to, then one would expect a comparatively high percentage of failures in those patients with cervicitis. The results of our investigation however show that this is not so The most likely explanation is that when both metronidazole and nifuratel have failed the organisms have developed an increased resistance. It is possible that simultaneous treatment with several preparations could prevent this development.

A fungal vaginitis was noted in 29 per cent of patients following successful treatment of trichomonas vaginitis with metronidazole (*Keighley* 1962) but one can presumably ignore this complication with nifuratel as this drug has a fungicidal effect. This effect is being studied by us and the results will be published later

Conclusion

We may presume that trichomonas vaginalis like other microorganisms is capable of adapting itself gradually making antibiotics less effective. The results of our investigation confirm the results of other workers namely that nifuratel provides a valuable addition to the medical armamentarium, with a cure rate of 77 per cent.

SUMMARY

A new trichomonicidal preparation, nifuratel (Polmiror[R]) has been evaluated in 95 patients with trichomonas vaginalis vaginitis. The treatment consisted of local application for all the patients for 10 days 60 patients received oral tablets for 7 days and 30 patients for 10 days. The results in both groups were equally good, a cure rate of 77 per cent. Factors such as parity the duration of symptoms, prior treatment and hormonal status did not influence the results A co-existent cervicitis did not decrease the cure rate significantly Simultaneous treatment of the sexual partner improved the results. Combined therapy with other medicaments may further improve the cure rates.

Table VI. *Results of Treatment with Nifuratel in Relation to Other Treatment*

Previous Therapy	Nifuratel 1 Course			Metronidazole 1 Course		
	N	Cured	Not Cured	N	Cured	Not Cured
Not cured with nifuratel				18	7	9
Not cured with metronidazole	9	4	5			
Not cured with other treatment	11	7	4	3	1	1

1 Two patients were not controlled.
2 One patient was not controlled.

them only after the second course. One patient developed nausea during treatment. No side effects were serious enough that the treatment had to be withdrawn and none of the patients considered the concomitant use of oral and local administration a serious inconvenience.

Discussion

A long term assessment of the true effect of treatment is always confused because it is often impossible to discriminate between recurrence and reinfection. "Nonspecific vaginitis" is not included in this investigation because in such cases one often suspects that trichomonas vaginalis is the cause but it cannot be demonstrated. A cure rate of 77 per cent as the result of treatment with nifuratel must be judged as good. We have not been able to achieve results as good as those previously published, but the difference is not very great and could depend upon, among other things, different methods of selection and different lengths of follow up time. For example, our results follow closely those shown in Table I if one compares them with those investigations where the results after the first to third month of observation are shown.

It can be seen from our investigation that if treatment with metronidazole failed, then the results with nifuratel might be expected to yield approximately a 50 per cent cure in the treated patients and the same conclusion can be drawn if the prepara

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TREATMENT OF CANDIDAL VAGINITIS WITH NIFURATEL

BY

J. CHR. AURE AND H. GJØNNAESS

Candidiasis is one of the most common causes of vaginitis and vulvitis. Candida albicans is commonly found but Candida stellatoidea, Candida tropicalis and Candida krusei occur not infrequently (*Hurley* and *Morris* 1964 *Winner* and *Hurley* 1966) Not all species of Candida are necessarily pathogenic and they may be present as saprophytes in the vagina. Earlier investigators reported Candida in 1–41.5 per cent of cases (*Haberman et al.* 1962 *Rohatiner* 1966 *Purola et al.* 1967 *Robinson et al.* 1967) The aetiological factors in candidal vaginitis are not yet fully elucidated. However it has been observed that female diabetics, pregnant women and women on broad-spectrum antibiotics or steroids are particularly prone to infection (*Jennison* and *Llywelyn-Jones* 1957 *Winner* and *Hurley* 1966) Recently a high incidence has been reported in women taking hormonal contraceptives (*Catterall* 1966)

The impression that vaginitis is more often caused by Trichomonas than by Candida has changed in recent years and the reverse appears to be true in many countries (*Read* 1962 *Rohatiner* 1966) *Pace* and *Schantz* (1956) identified Candida seven times more often as Trichomonas in non-pregnant women with vaginitis and 15 times as often in pregnant women. Mixed trichomonal-candidal infection is relatively common, particularly in pregnant women (*Lang et al.* 1962 *Alteras et al.*, 1965) However Candida may easily be overlooked in wet smears examined by direct microscopy if Trichomonas also is present.

Acknowledgement

The authors wish to express their thanks to Mr Ake Nilson, AB Bofors Nobel Pharma for valuable advice and technical help and also to AB Bofors Nobel-Pharma who have provided us with this new preparation.

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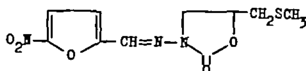
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J CHR. AURE AND H. GJØNNAESS

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5-[(methylthio)methyl]-3-
[(5-nitrofurfurylidene)amino]-
2-oxazolidinone

Fig 1 Nifuratel

In contrast to Trichomonas which is also found in the cervix and urethra candidiasis is usually confined to the vagina and vulva. Local treatment should therefore be effective. Various methods of treatment have been used, such as douching with alkaline solutions and painting with gentian violet resulting in cure in about 60 per cent of cases (*Jennison* and *Llywelyn-Jones* 1957) More recently various antibiotics such as Candicidin and Chlordantoin, have been applied locally the reported cure rates ranging from 50 to 75 per cent (*Nathanson* 1960 *Mendel* and *Bone* 1961 *Kantor et al.*, 1966) It has been reported that Nystatin results in cure in 60–100 per cent of cases (*Pace* and *Schantz* 1956 *Charnock* 1958 *Kantor et al* 1966)

Nifuratel is a new drug, synthesized by Polichimica SAP of Milan. It is a derivative of nitrofuran, its generic name being Nifuratel It will be available as Polmiror[R] in the Scandinavian countries. Fig 1 shows its chemical configuration In a previous trial (*Gjonnaess* and *Aure* 1969) combined local and systematic treatment also proved to be effective against trichomoniasis. It has previously been studied in Spain by *Macia* and *Dexeus* (1966) and in Japan by *Magara* (1967) the former workers achieving cure in 23 out of 33 cases of candidal vaginitis and the latter in 28 of 41 cases.

Material and Method

Nifuratel was tested from March 1966 to May 1967 on 60 patients with candidal vaginitis In each case fungus was identified in wet

Table I. Results of Treatment with *Nifuratel in the Present Series*

	1st Course			2nd Course			Cured after 2nd Course	%
	No. of Patients	Cured	Improved	No. of Patients	Cured	Improved		
Pregnant	16	11	5	4	2	2	13	81
Non-pregnant	44	35	8	3	1	2	36	82
Total	60	46	13	7	3	4	49	82

One patient did not return for follow-up examination after th 2nd course.
One patient did not return for follow-up examination.
One patient did not follow the instructions for treatment properly but her condition had improved. Two *patients* were well subjectively *but microscopy* revealed solitary mycetes. The condition of tw patients had improved after the 1st course None of these patients returned for follow-up examination after the 2nd course.

smears by direct microscopic examination. Sixteen patients were pregnant, one patient was taking hormonal contraceptives and two patients had gonorrhoea in addition to candidiasis. Three patients were menopausal when the first symptoms of the infection appeared. None of the patients had diabetes. Two vaginal tablets each of 0.25 g Nifuratel were inserted daily one in the morning and the other in the evening, for 15 days treatment was discontinued during menstruation. Follow-up examination was carried out 10 days or more after completion of treatment. Patients who were found to be free from symptoms and who had a *pale* healthy vaginal epithelium and normal vaginal secretion as judged both macroscopically and microscopically were considered cured. If the first course of treatment did not result in cure a second course was given following the same regime as *described above* This second course was required in 13 cases. Seven patients in this latter group returned for follow-up examination after the second course of therapy Regular follow-up examinations over a prolonged period of time were not possible be se the patients lived at a great distance from the hospital but each patient was instructed to attend if she noted the slightest symptoms of recurrence of the infection.

Results

The results of treatment are shown in Table I. Treatment was effective in 49 out of the 60 patients in this series (82 per cent) There was no difference in the response in pregnant and non pregnant women The patients who were not completely cured showed marked improvement, both subjectively and objectively

Four patients had mixed trichomonal-candidal infection. They were first treated with metronidazole and thereafter with Nifuratel, the treatment resulting in cure Of 5 patients who had been treated unsuccessfully with Nystatin, 4 were cured following a course of Nifuratel and the condition of the fifth patient, who was pregnant improved markedly

No side-effects were detected

SUMMARY

A new drug, Nifuratel (Polmiror[R]) was used for the treatment of vaginal candidiasis in 60 women, 16 of whom were pregnant Two vaginal tablets each of 0 25 g Nifuratel were inserted daily one in the morning and the other in the evening, for 14 days. Both pregnant and non-pregnant women responded equally well to this therapy The results were as follows 46 patients were cured after one course of treatment and a further 3 patients following a second course the cure rate thus being 82 per cent The condition of the other 11 patients improved markedly The drug proved to be a valuable therapeutic agent in cases of candidal vaginitis.

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ULTRASTRUCTURE OF HUMAN CHORIOCARCINOMA

BY

M. KNOTH H HESSELDAHL AND J FALCK LARSEN

The ultrastructure of normal human trophoblast has been de-scrled by several investigators (*Larsen* 1963) However observa tions on malignant trophoblast are few because choriocarcinoma is a rare tumour *Wakitani* (1962) dealing with one patient obtained material by hysterectomy and from metastases. *Onoé* (1962) has a single illustration showing characteristic features of choriocarcinoma in a review on the electron microscopy of human malignant growths Because of the lack of clinical material, trans plantable tumour carried in the hamster cheek pouch has been studied by *Wynn* and *Davies* (1964) and in hamster liver by *Larsen et al* (1967)

During the latter study many strange phenomena were observed which had not been described in previous papers on human biopsies When the opportunity presented biopsies were obtained by us from two patients with choriocarcinoma and this tissue was compared with the transplantable tumour

Material and Methods

Tissue was obtained from two patients with choriocarcinoma and from 26 hamsters with transplanted human choriocarcinoma n serial cheek pouch passage

The tissue from the patients was obtained and treated as follows.

Case one· A curettage performed n a 32 year-old woman revealed a hydatidi

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form mole. Spotting recurred, and two months later a second curettage was carried out. Choriocarcinoma was suspected. The diagnosis was confirmed by hysterectomy. Two weeks later metastases were observed in the vagina and biopsies were obtained for electron microscopy. The tissue was fixed in osmic tetroxide and embedded in Durcopan®. The patient was treated with Methotrexate® and has now survived for two years. Chorionic gonadotropin (HCG) has not been detected in the urine since chemotherapy was completed.

Case two. A 29-year-old woman was admitted to the Municipal Hospital in Copenhagen (Kommunehospitalet) because of vaginal bleeding during the fifth month of pregnancy. The haemorrhage became excessive and as the cervix remained closed, the uterine cavity was emptied by abdominal hysterotomy. A hydatidiform mole was found and removed. As the patient continued to excrete chorionic gonadotropin for six months hysterectomy was performed. A small tumour was found in the fundus, and microscopic examination revealed that it consisted of cytotrophoblast. Cytotrophoblast was also found in the parametrial vessels. Tissue was fixed for electron microscopy in osmic tetroxide and embedded in Vestopal®. The patient was treated with four courses of Methotrexate® but suffered severe pain, became emaciated and died. No autopsy was performed.

The transplanted tumour. This tissue was obtained during experiments in which the Greene choriocarcinoma (*Hertz* 1961) was carried by hamster cheek pouch transfer approximately every ten days. The experiments were done to study the relation between tumour and invaded tissue (Larsen et al. 1967). The tissue was fixed in osmic tetroxide and embedded in Epon®.

The ultrathin sections were cut on Porter Blum and LKB-ultramicrotomes, stained with lead citrate and uranyl acetate, and examined in a JEOL JEM-6 C electron microscope.

Results

Case one Compared with the Transplanted Tumour

The fine structure of the choriocarcinoma from the first patient and from the cheek pouch transplanted Greene tumour was identical. Both consisted mainly of two types of cells. One type (Fig. 2) resembled the cytotrophoblast of the early human placenta while the other type was a giant cell with many characteristics of the normal syncytiotrophoblast (Figs. 3 and 5).

The first type of cell, *the cytotrophoblast*, was regular in shape usually elongated or spindle-shaped. The nucleus was large with an irregular nuclear membrane often with infoldings (Fig. 2). The nucleolus was skein-like and rather large. The cell membrane

was regular with few indentations but in some places microvilli of variable form and amount were found. They were often short and clubbed and sometimes branching In this type of cell very limited endoplasmic reticulum could be found. The RNA was present in the form of clusters of ribosomes lying free in the cytoplasm. However in some cells the ribosomes were found as part of the sparse endoplasmic reticulum (Fig 6) The golgi apparatus was abundant in many cells. In some a large part of the cytoplasm was occupied by accumulations of smooth golgi membranes and vesicles (Fig 7) The mitochondria measuring about 0.5 to 3 μ appeared slightly larger than those of normal trophoblast. They were numerous many had a polygonal form with few curled cristae and abundant matrix of medium electron density (Figs 2 and 8) but mitochondria of more ordinary shape were also found (Fig 8)

The cytoplasm contained variable amounts of agranular reticulum and vacuoles (Fig 2) some containing fine granular substance Occasionally centrosomes were found (Fig 7)

The other cell type *the giant cell* (Figs 3 and 4) measured more than 100 μ in diameter The nucleus was in a central position in most of the cells The *nuclear membrane* (Fig 9) was very irregular with deeper infoldings than those of the cytotrophoblastic cell type (Fig 2) Many of the nuclear protrusions were branching and the nuclear pores were rather large up to 1000 A in diameter (Fig 9) This cell type was identified as the multinucleated type in the light microscopic sections but only occasionally was more than one nucleus found in the ultrathin sections. The cell membrane often had more slender microvilli than those found on the surface of the cytotrophoblast. At the base of the microvilli small vesicles indicated pinocytotic activity (Fig 3) The endoplasmic reticulum of this type of cell was of the rough-surfaced type and consisted of numerous short channels or vacuoles (Figs. 3 and 4) The channels were often found to be slightly dilated and somewhat irregular in outline Polyribosomes free in the cytoplasm were also found In some cells endoplasmic reticulum of the common type was observed (Fig 9) The mitochondria of the giant cells (Figs. 3 and 4) were relatively smaller and less numerous and they were more regular in shape



Fig. 1. Portion of cytotrophoblastic cell from case two. Many club shaped microvilli on the surface (MV). The mitochondria (M) have few cristae. In the cytoplasm large inclusions (I) are found. NC, Nucleus. ×12,500

Fig. 2. Portions of two cytotrophoblastic cells from the transplanted tumour. The nucleus (NC) is very irregular. The mitochondria (M) are large, irregular in shape and have few cristae. Inclusions (I) are numerous. The cells are connected by desmosomes (D). ×8000

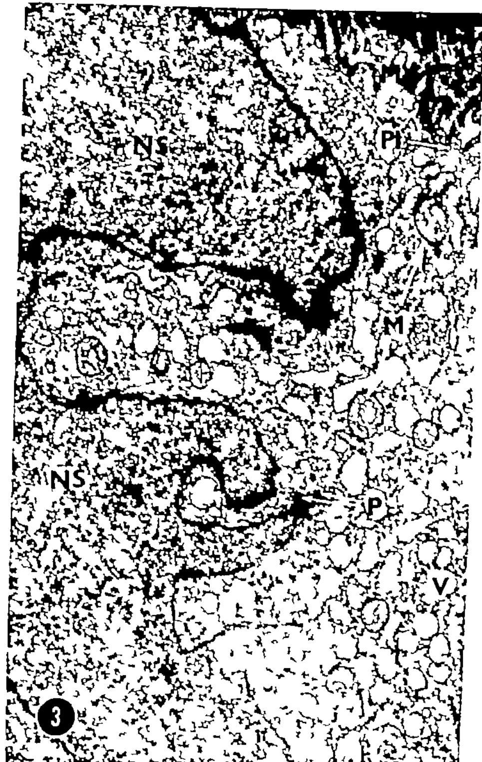


Fig. 3 Portion of syncytial type t tumour ell f m transplanted tumour The nucleus (NS) is very larg w th nf lding Th ytoplasm conta s many vacuoles (V) Some t the bosomes ar tt hed t these acuoles P- Nuclear pores, M Mitoch na Th ell membrane possesses mi villi



Fig 4 Giant cell from case one The mitochondria (M) have few cristae NS Nucleus The cytoplasm is rich in golgi membranes (G) MY Myelin Figure. L Lipid granule. LY Lysosomes ER Endoplasmic reticulum ×28,000
Fig 5 Syncytiotrophoblast of transplanted tumour containing lacunae (LA) NS. Nucleus of syncytiotrophoblast NC Nucleus of cytotrophoblast ×2000



Fig 6 Portion of cytotrophoblastic ll f om case one Large accumulation of endop.asmic reticulum (ER) G Golgi apparatus M Mitochondrion. R. Ribosomes. ×32,000

Fig 7 Portion of cytotrophoblasti cell from ase one M. M tochondrion AR. Agranular reticulum. G Golgi ppar tus ER. Endoplasmic reticulum C. Centrosomes MI. Mitochondrion of mor normal shape. F Fibrils of intercellular substance. ×28,000

Fig. 8 Mitochondria from cytotrophoblast of transplanted tumour. M. Large, star shaped mitochondrion with few cristae. MI. Mitochondrion of ordinary type. ×18,000

Fig. 9 Portion of syncytiotrophoblast from case one. The nucleus (NS) has very deep infoldings. The nuclear pores (NP) are large. ER. Endoplasmic



Fig. 10 Syncytiotrophoblast from transplanted tum exhibiting phagocy tosis. NL. Nucleus of phagocytosed l er l In omplete membranes a found between *cytoplasm f liver ll (CL) and ytoplasm of trophoblast* (CS) NS Nucleus of troph blast LY Lysosomes 10 000

Fig 11 Ghost cell f om case one NP N leus of phagocytosed cell Membrane between *the phagocytised cell and the ytoplasm of the trophoblast* (CS) is broken in many places (White ar ow) CP· Cytoplasm of phagocytosed cell. ×11 000



Fig. 12. Myelin figure (M) and mitochondrial like figure (White arrow) inside the nucleus of tumour cell from case one. Black arrows indicate nuclear membrane ×22,000

Fig. 13 Unidentified structures in the cytoplasm of trophoblastic giant cell of case one. In some of these the internal structure resembles that of desmosomes (White arrows) ×70,000

Fig. 14 Intracellular desmosome in giant cell from case one. NS. Nucleus D. Desmosome. ×38,000



NP
CP
CS
11

Fig 10. Syncytiotrophoblast from t ansplanted tumour exhibiting ph gocy tosis. NL. Nucleus of phagocytosed li er ell In omplete membranes are found between cytoplasm of l er ell (CL) and ytoplasm of trophoblast (CS) NS Nucleus of trophoblast L\ Lysosomes 10 000

Fig 11 "Ghost cell f om case one NP Nucleus of phagocytosed cell Membrane between the phagocytised cell and the ytoplasm of the trophobla t (CS) is broken in many places (White arrows) CP Cytoplasm of phago-cytosed cell. ×11 000



NS

NS

14

Fig. 12. Myelin figure (M) and mitochondrial-like figure (White arrow) inside the nucleus of tumour cell from case one. Black arrows indicate nuclear membrane. ×22,000

Fig. 13. Unidentified structures in the cytoplasm of trophoblastic giant cell of case one. In some of these the internal structure resembles that of desmosomes (White arrows). ×70,000

Fig. 14. Intracellular desmosome in giant cell from case one. NS: Nucleus. D: Desmosome. ×38,000

with more cristae than those described in the cytotrophoblastic cells. In many cells lysosomes and myelin figures were observed (Fig 4)

Some giant cells had additional ultrastructural features. Intracellular irregular canaliculi or lacunae were found in some of the giant cells (Fig 5) The spaces were lined with microvilli, and contained an agranular substance of low electron density

Phagocytic activity was seen in several giant cells (Fig 10) The cytoplasm was more or less filled with partly digested cellular material. Sometimes this material was surrounded by a shadow like membrane retaining some of the characteristics of a Phagocytosed nucleus (Fig 11) In other regions of the cytoplasm collections of amorphous material of medium electron density with incomplete surrounding membranes were seen (Fig 1)

In many areas bundles of collagen with characteristic periodicity were observed. In other areas the intercellular substance was rich in fibrillar material of high electron density almost completely surrounding the tumour cell. Sometimes the fibrils were condensed close to the cell membrane simulating a basement membrane.

In some of the cells intranuclear bodies were observed. One of these was a structure resembling a myelin figure surrounded by a condensation in the nuclear substance and in the same nucleus a mitochondrion like structure was also found (Fig 12) In other nuclei the intranuclear body consisted of a granular substance surrounded by a membrane.

Desmosomes were observed between both types of tumour cells In the transplanted tumour desmosomes were found also between the tumour cells and the host tissue (Larsen et al 1967) In one of the cells from case one was found an intracellular desmosome (Fig 14) In other cells from the same tumour the cytoplasm contained unidentified desmosome-like figures (Fig 13) Desmosomes connecting tumour cells with host cells were not convincingly shown in the tissue from either case one or case two

Almost all cells could be classified as cytotrophoblastic or giant cells (syncytiotrophoblast) However a few cells had some of the characteristics of both and may represent a transitional

type Other cells differed by having an extraordinarily high content of golgi elements (Fig 10 in *Larsen et al.* 1967)

Case two The tissue obtained from the second patient consisted of the cytotrophoblastic type only This type (Fig 1) was similar to the corresponding element in the tumour from case one and in the transplanted tumour The mitochondria had the same simple appearance with few cristae Almost all the cells had numerous microvilli. Between the microvilli vacuoles beneath the cell membrane indicated pinocytotic activity The cytoplasm was rich in large inclusions (Fig 1) consisting of fine granular substance bordered by an incomplete membrane

Discussion

The biopsies from case one showed the typical plexiform pattern of trophoblast, and the admixture of blood clot and fragments of necrotic decidua, diagnostic of choriocarcinoma (*Hertig* and *Mansell*, 1956)

Benirschke and *Driscoll* (1967) stated that choriocarcinoma is always composed of both elements of trophoblast syncytium and cytotrophoblast. Only cytotrophoblast was found in the sparse material from the second patient, and there was no opportunity to obtain additional biopsies. *Novak* and *Seah* (1954) also found both elements in the examination of biopsies from 74 cases registered in the Mathieu choriocarcinoma registry but in one case a great preponderance of cytotrophoblastic cells was clearly evident. In our second case the clinical history leaves no doubt that the patient died from choriocarcinoma. Moreover the ultrastructure of the cytotrophoblast from that patient was very similar to that of the cytotrophoblastic cells of case one and the transplanted tumour

The similarity in the ultrastructure of transplanted tumour and tissue obtained from the patients confirms on the submicroscopic level the observations by Hertz (1961) who found no change in the morphology of choriocarcinoma during its transfer from man to experimental animals.

In many ways the ultrastructure of tumour resembled that of normal placental trophoblast. The majority of cells could be

classified into two groups according to their resemblance to either cytotrophoblast or syncytiotrophoblast of the normal placental villus.

The first type differed from normal first trimester cytotrophoblast primarily in the size of the cell and in the irregularity of the nucleus and mitochondria The cytotrophoblast of the human placenta measures about 20×50 μ but *the malignant cytotrophoblast* often measures 100 μ or more in diameter The malignant nuclei are larger and the nuclear membrane is more irregular

The second type *the giant cell* resembled the syncytiotrophoblast of the early normal placenta (*Boyd* and *Hughes* 1954 *Wislocki* and *Dempsey* 1955 *Ter akis* 1963 *Lister* 1964 *Anderson* and *McKay* 1966), but differed in the appearance of the nuclei These were much larger with complicated infoldings. The nuclear pores measured about 1000 Å, which is about twice the normal size (*Freeman* 1964 *Fawcett* 1966)

No single morphological characteristic is specific of cancer cells but many features found in choriocarcinoma are similar to those observed in other types of malignant cells. Two of these features are nuclear hypertrophy and irregularity The deep indentations of the nuclear membrane increase the surface of the nucleus Therefore the possibility of facilitating nucleo-cytoplasmic interchanges must be considered. The large size of the nuclear pores may have the same consequence Although rare nuclei with irregular nuclear membranes can sometimes be found in the normal syncytiotrophoblast (*Wislocki* and *Dempsey* 1955)

Cancer cells generally have a simpler ultrastructure than fully differentiated normal cells (*Oberling* and *Bernhard* 1961) In our study however the tumour cells retained many of the cytoplasmic characteristics of the normal trophoblast.

The golgi apparatus was prominent in some of the cells, especially in those of the cytotrophoblastic type A prominent golgi apparatus has been found in mouse mammary tumours (*Bernhard* and *Guérin* 1957) but generally golgi elements are not conspicuous in malignant cells (*Oberling* and *Bernhard* 1961) The mitochondria were irregular in shape with few curled cristae often having a swollen appearance This type of mitochondrion

was found to be the feature which usually distinguished the tumour cells from normal trophoblast. This swollen type of mitochondria with few cristae has been described in many types of tumour (*Oberling* and *Bernhard* 1961) Unsatisfactory fixation cannot explain this appearance of the mitochondria as other cell organelles were adequately fixed, and normal mitochondria were also found in the connective tissue and liver cells in the same sections.

The amount of endoplasmic reticulum in cancer cells tends to decrease with dedifferentation of the tumour cell (*Oberling* and *Bernhard* 1961) Only sparse lamellae can be observed and most of the RNA-granules are found lying free in the cytoplasm. This picture is seen in the majority of cytrotrophoblastic cells in all the investigated choriocarcinoma (*Midgley* and *Pierce* 1963 *Wynn* 1963) The syncytiotrophoblastic cells on the other hand, could be recognized by a cytoplasm rich in endoplasmic reticulum which is characteristic of syncytial trophoblast of the normal placenta. This type of endoplasmic reticulum consists of dilated sacs or vacuoles with a homogeneous content of medium dense material.

Much effort has been made to define the term choriocarcinoma The clinician would prefer a classification of trophoblastic growths indicating the prognosis. In most cases, patients without chemotherapy due within two years. However *Brewer et al.* (1961) reported that of 147 patients noted in the Mathieu register as cases of choriocarcinoma treated by surgery and supplemented in two instances by irradiation, 21 survived more than five years. The tissue from these 21 patients was studied carefully by the authors as well as by *Hertig* (1961) but they could not point out any morphological criteria which could separate the 15 per cent of curable lesions from the 85 per cent which killed the patients despite treatment.

Due to this lack of light microscopic criteria it would be of great value if a study of the ultrastructure could give clues to the prognosis. The degree of cytoplasmic simplicity and the mitochondrial deformity may be indicative of increasing malignancy Only a submicroscopic examination of biopsies from many patients with choriocarcinoma and a clinical follow-up study might show

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if these criteria can be used. However this would be difficult because of the rarity of the disease in most parts of the world. Furthermore it may not be possible to use such criteria even with electron microscopy if the variations in clinical course are caused by host differences rather than by variations in tumour malignancy

Choriocarcinoma produces gonadotrophic hormones Using the fluorescent antibody technique evidence has been presented favouring the theory that HCG is produced in the syncytiotrophoblast (*Midgley* and *Pierce* 1962) This view is supported by the presence of abundant endoplasmic reticulum in the syncytiotrophoblast in contrast to the sparse amount in the cytotrophoblast.

It is difficult to distinguish HCG biologically and chemically from the luteinizing hormone (LH) of the pituitary *Farquhar* and *Rinehart* (1954) in their studies of the rat pituitary found that castration resulted in the proliferation of signet ring cells with a large cytoplasmic vacuole and also of cells with a filigree-like cytoplasm due to innumerable irregular vacuoles. Basing their arguments on bio assays, these authors suggested that the former type of cell is the source of follicle stimulating hormone while the later is the source of luteinizing hormone It is interesting that cells similar to both types have been found in the choriocarcinoma transplanted to hamster liver (*Larsen et al* 1967)

Electron dense granules of the kind found in the pituitary have not been observed in the tumour nor are such granules found in the normal placental trophoblast. However in some of the tumour cells of both types fine granular material of medium electron density was seen sometimes enclosed within a membrane This material is well known from studies on the cytoplasm of early placental syncytiotrophoblast (*Terzakis* 1963) and may represent the ultrastructural picture of HCG

It is well known that viruses may cause leukaemia and other malignant growths. Because of the unknown aetiology of chorio carcinoma this tissue was examined to detect possible evidence of virus No virus like particles or crystals were found However virus affected tissue may not show any specific changes. The cell injuries may only include enlargement of the nuclei nucleolar

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found phagocytosis in some areas especially in fields with considerable destruction of tissue and some of the phagocytized cells were blood cells The limitation of phagocytosis to a few fields could be explained by the assumption that choriocarcinoma cells phagocytosed devitalized material only The devitalization could have resulted from lack of blood supply or by toxic agents from the invading tumour

In a previous publication (*Larsen et al.* 1967) desmosomes between choriocarcinoma and the invaded tissue were described. The presence of desmosomes between trophoblast and liver cells is a strange phenomenon The cells they connect are not only from different organ systems but also from different species man and hamster

Another strange observation is the presence of intracellular desmosome-like figures found either as small groups (Fig 13) or interposed between two different nuclei is one cell (Fig 14) *Onoé* (1962) has in a study of the ultrastructure of human choriocarcinoma a single micrograph showing intracellular desmosome-like structures. Recently *Okudaira* and *Strauss* (1967) described aggregations of similar structures in chorioadenoma destruens. If these elements are desmosomes they may originate from disintegrating cell membranes between cytotrophoblastic cells as they merge into a syncytium or from the membranes of phagocytozed cells.

SUMMARY

Tumour tissue from two patients with choriocarcinoma and tumours transplanted into hamster liver were studied with the electron microscope The ultrastructure of choriocarcinoma is compared with that of other cancer cells and with non-malignant trophoblastic tissue

Two types of trophoblastic tumour cells were observed. The first type resembled the cytotrophoblast of normal placenta but the cells were larger and contained large irregular mitochondria with few cristae The second type resembled the normal syncytiotrophoblast, but differed in the large irregular nuclei

The phagocytic activity the possible ultrastructural picture of

chorionic gonadotrophin and the existence of intracellular spaces lined with microvilli are discussed. In some of the cells, nuclear inclusions and intracytoplasmic desmosome-like figures were observed. As to the aetiology of choriocarcinoma, no conclusive evidence of viral origin could be demonstrated.

Addendum

Since this manuscript has been submitted, *Inferrera et al.* (Arch. Ostetr e Ginec. 72 707 1967) have published a paper on the ultrastructure and histochemistry of choriocarcinoma. They found an intermediate type and an extremely undifferentiated type of cell besides the cytotrophoblast and syncytiotrophoblast. The description of the ultrastructure is in good agreement with that presented in this paper These authors describe numerous osmiophilic granules in the syncytium and they consider these to be a possible morphological expression of the secretion of chorionic gonadotropins

Acknowledgement

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PERCUTANEOUS CATHETERIZATION OF THE HYPOGASTRIC ARTERY IN LOCAL INFUSION THERAPY WITH CYTOSTATICS

BY

INGMAR FERNSTRÖM, KARL-GUSTAV MELIN AND NILS WIQVIST

In diagnostic pelvic arteriography using compression of the femoral artery there is no need for selective catheterization of the hypogastric arteries. However local infusion therapy with cytostatics in cases of advanced cervical carcinoma or malignant trophoblastic disease requires introduction of thin catheters into the hypogastric arteries. It is advantageous for many reasons if these catheters can be introduced without subjecting the patient to distressing surgical procedures. It is also necessary that the catheters be maintained in proper position during a comparatively long period of time, with a minimum of discomfort for the patient and without causing complications.

Some authors have introduced the catheters by extraperitoneal surgical exposure of the hypogastric arteries (*Cahill* and *Zeit* 1961 *Hulka* and *Bisel* 1965 *Sullivan et al.*, 1960 *Trussel* and *Mitford Barberton* 1961) whereas others have introduced the tubes in the gluteal arteries (*Laufe*) Several investigators have given local infusion of methotrexate after percutaneous puncture of the femoral artery and introduction of a catheter into the lower aorta (*Bateman et al.* 1966 *Botman et al.* 1956 *Krakoff* and *Sullivan* 1958) The first two methods mentioned have the drawback of necessitating a major surgical procedure, whereas

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the last distributes the cytostatic agent into both the external and internal iliac arteries. It would be of advantage if thin polyethylene catheters could be introduced into the hypogastric arteries by the percutaneous technique in the same way as in selective diagnostic arteriography of the renal, coeliac and mesenteric arteries In these roentgen examinations the catheter is introduced percutaneously and the tip manoeuvered into position under roentgen television control. This technique for catheterization of the hypogastric artery has so far been used to a very small extent. *Michaelsson* 1965 described a new method by which large branches of the aorta could be catheterized easily After some modification this technique could also be used for catheterization of the hypogastric artery However the large diameter of the catheter (PE 240) precluded its retention in the artery for the desirable period of time Therefore in a few cases a thin teflon catheter was introduced via the large polyethylene catheter which was subsequently withdrawn. *Wholey* and *Jackman* 1966 reported on a technique by which the catheter could be manoeuvered into the hypogastric artery by a special instrument. However the catheters used had a rather large diameter of more than 2 mm. *Nilsson* 1967 publihed a report of 30 patients in whom one or both hypogastric arteries had been catheterized by an Ödman catheter with bent tip and with an external diameter of 2.2 mm. These techniques have been used for the diagnosis of urinary bladder tumours but tubes of such large diameter and stiffness can hardly be left in the femoral and hypogastric arteries over the period of 7–10 days which is considered as a minimum for infusion of cytostatics.

A method for percutaneous catheterization of the hypogastric artery was recently described by *Onnis et al* 1967 These authors suggested the use of Ödman catheters with a small diameter for selective catheterization of the hypogastric arteries. However even the thinnest catheters of this type are comparatively stiff and have an outer diameter of 1 8 mm.

During 1965 in a few cases we used Michaelsson s method to introduce thin catheters However the technique was found to be difficult and time consuming and often failed. In 1966 a new method was introduced which was easy and reliable.

Technique

Introduction of the catheter was made according to the Seldinger technique (*Seldinger* 1953) Needle no. PE 160 guide no 100 and polyethylene catheter no PE 100 were used. This catheter has an outer diameter of 1.52, an inner diameter of 0.86 and a length of 500 mm. In preparing the catheter the tip is tapered and the other end formed into a collar in the usual way In addition, the tip of the tubing is heated by *steam* and bent into a loop as shown in Fig. 1

Fourteen different roentgen-television combinations were tested for this purpose but most of these were unsatisfactory since the catheter could not be adequately visualized. The roentgen-television equipment finally chosen has *high gain combined with* good resolution of fine details. This television apparatus is equipped with a vidicon tube which has longer persistance than an orthicon or a plumbicon tube. However we have not encountered any difficulties in following the catheter tip during the manipulations.

To improve the picture quality the technique of geometric magnification was used during the catheterization. With the image intensifier input screen placed at a distance of about 50 cm from the patient a linear magnification of 2 1 is achieved. This has two advantages. In the first place image resolution is substantially increased, since the ability of the roentgen-television equipment to display small details is primarily determined by the number of television scanning lines devoted to the detail in question. (In view of the limited resolution permitted by roentgen-television, the effect of focal-spot penumbra is, *as compared* with roentgenography of secondary importance.) In the second place contrast is much improved. The air gap between the patient and the image intensifier removes almost all the stray radiation, which is not directive Removing the disturbing stray radiation background not only improves contrast but also permits more effective utilisation of the electronic amplification in the television chain the patient dose has therefore to be increased by a factor f only two instead of the theoretical factor that one

Manufactured by SAAB Jonköping, Sweden

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Table I. Measured Roentgen Dose at the Image Intensifier Input (A) and at the Hands of the Examiner (B) during Hypogastric Catheterization. The Tube Voltage was 65 kV and a Geometric Magnification of 2:1 was used. Roentgen Field Size at Intensifier Input was 12×12 cm

Centre of Picture at	A mR/h	B mR/h
Th_3	9.5	0
L_1	7.5	0.2
L_5 S	7.0	0.6
Small pelvis	6.0	3.0

tip of the catheter to regain its original loop shape (Fig. 2 A). This can be accomplished by pushing and pulling the catheter until its tip is hooked into one of the branches of the aorta (Fig. 2 B). On pushing the catheter further it regains its original loop form, with the tip directed downwards (Fig. 2 C). In some cases this takes place spontaneously without manipulation, when the guide is withdrawn. The tip of the catheter is then withdrawn downwards to the level of the aortic bifurcation, care being taken that the tip is not introduced into some other branch of the aorta. The tip must be directed laterally (Fig. 2 D) when passing the bifurcation. The loop of the catheter is then easily withdrawn into the upper segment of the common iliac artery. Injection of contrast medium then gives information on the origin of the hypogastric artery (Fig. 2 E). The tip is then brought to this level and manoeuvered into the hypogastric artery by axial rotation (Fig. 2 F). Further axial rotation and retraction will finally bring the catheter in proper position (Fig. 2 G).

Results

Up to the present time 19 patients have been catheterized bilaterally and two unilaterally totalling 40 catheterizations. There have been no unsuccessful attempts. Six patients suffered from choriocarcinoma, 13 had a recurrent cervical cancer and one cancer of the vulva.

The procedure has not met with technical difficulties and the



Fig. 1 The loop-formed tip of the catheter *Right* Before introduction into the artery *Left* After introduction into the aorta. During this procedure the catheter has been stretched out on the guidewire resulting in reduction of the loop form

might expect to be required by 2 1 geometrical enlargement. The catheterizations were carried out with a tube voltage of about 65 kV and a current of 0.2–0 6 mA.

Since the roentgenologist has to work closer to the roentgen field than during catheterizations of other arteries it is important that the level of stray radiation be kept as low as possible. Therefore the roentgen television assembly must be highly sensitive, the smallest possible roentgen field must be used and the roentgen tube must be placed under the examination table. The stray dose received by the examiner under these conditions are given in Table I

The femoral artery is punctured in the groin after infiltration with local anaesthetic The catheter is then introduced into the aorta using the Seldinger technique and visualized on the monitor by filling with 76 % Urographin To introduce the catheter into the hypogastric artery the tip has to be turned downwards. Generally, the diameter of the aorta is too small to permit the



Fig. 3 39-year old woman with malignant trophoblastic tumour of the uterus. *A* Arteriography before treatment. The uterus is enlarged and hypervascular. Large cavities and early filling of veins are seen, indicating tumour infiltration of the uterine wall.

short periods. Continuous infusion of Methotrexate was given during 6–12 days by an electrical pump.

Fig. 3 illustrates a case with malignant trophoblastic tumour confined to the uterus and examined by arteriography before and after local infusion treatment. In this patient HCG tests soon became negative and complete regression was shown on the arteriograms.

There have been no clinical signs of thrombosis or other vas-

Bowman Infusion Pump Model 2, Process and Instruments Corporation, Brooklyn N.Y. U.S.A.

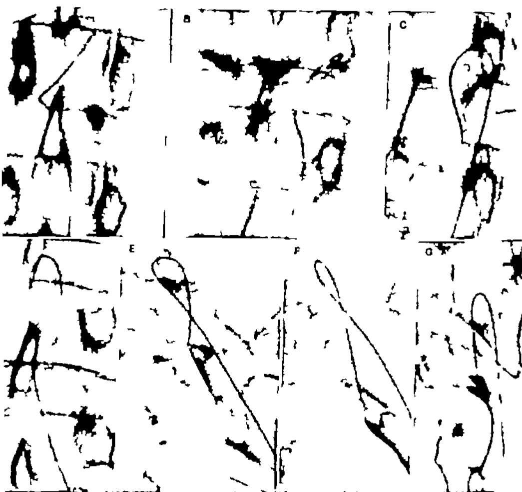


Fig 2. Series of X ray films illustrating manipulation of the catheter *A*. The catheter in the aorta with the tip directed upwards. *B*. The tip is hooked into an aortic branch C After manipulation the tip is turned downwards. *D* Before introduction into the common iliac artery the tip is turned laterally *E* Injection of contrast medium to localize the hypogastric artery *F* The tip is introduced into the hypogastric artery *G* Catheter in proper position after final adjustment.

period of time required for the introduction has successively decreased. At present the whole procedure with bilateral catheterization takes approximately 60 minutes or less. The catheters are maintained in proper position by fixation in the groin with surgical tape. The patient is allowed to move comparatively freely in the bed, and in some instances even leave the bed for



Fig. 3 39-year old woman with malignant trophoblastic tumour of the uterus. A. Arteriography before treatment. The uterus is enlarged and hypervascular. Large cavities and early filling of veins are seen, indicating tumour infiltration of the uterine wall

short periods. Continuous infusion of Methotrexate was given during 6–12 days by an electrical pump

Fig. 3 illustrates a case with malignant trophoblastic tumour confined to the uterus and examined by arteriography before and after local infusion treatment. In this patient HCG tests soon became negative and complete regression was shown on the arteriograms.

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Fig 3 B Polyethylene catheters in proper position in the hypogastric arteries. Infusion treatment with Methotrexate (50 mg/day) during 6 days combined with intramuscular inject on of folic acid (6 mg every 6 hours)

cular complications In 4 instances arteriographic examinations of the pelvic arteries were carried out within one month after the end of the infusion therapy There was no evidence indicating damage to the arterial wall One patient, suffering from advanced cervical carcinoma died one week after removal of the catheters.

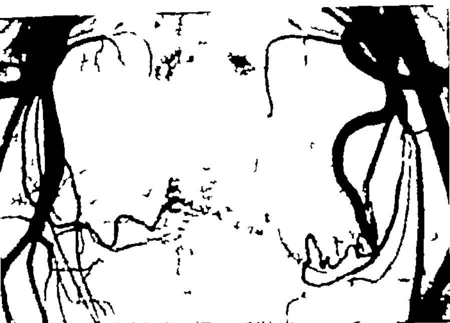


Fig. 3. C. Arteriography 2 months after treatment indicates normal appearance of the vascular anatomy

Autopsy showed only small thromboses in the iliac arteries (Fig. 4). The localisation and extension indicate that these alterations were due to mechanical damage to the intima caused by local pressure by the catheter. Such lesions will certainly heal and are evidently without clinical importance.

Discussion

The presence of a catheter within the large arterial stems during a long period of time may give rise to complications such as thrombosis, arterio-venous fistulas, aneurysm or haematoma. The catheter should therefore have a small diameter, a smooth surface and not be too rigid. Polyethylene satisfies these criteria and also has the essential property that it may easily be formed into

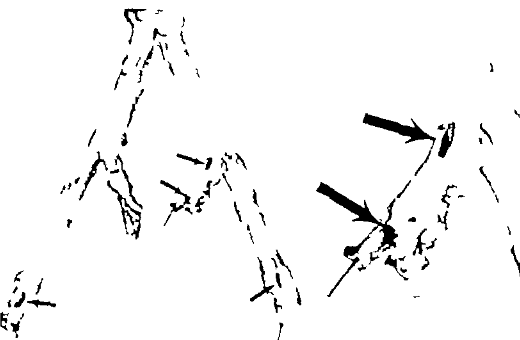


Fig 4 Autopsy specimen of the iliac arteries obtained from a patient with advanced cervical carcinoma one week after 7 days of bilateral hypogastric catheterization. The arrows indicate small thromboses within the distal part of the external iliac arteries and the left hypogastric artery The right photograph is a magnification of the left hypogastric artery

any desired shape after heating, contrary to most other synthetic materials.

The outer diameter and wall thickness of the polyethylene catheter determine the stiffness of the tubing It should be firm enough to manipulate both longitudinally and axially It should also largely regain its original loop-shaped form after withdrawal of the guide Moreover the inner diameter should be large enough to make possible visualization on the television-screen after in jections of contrast medium.

After some trials it was found that the most suitable catheter which met the above criteria corresponded to size no PE 100

The Seldinger technique is the method of choice for introducing the catheter since puncture of the femoral artery can be made with a needle of approximately the same outer diameter

as the catheter Seldinger needle no 160 is the smallest needle that fits catheter no. PE 100 The small calibre of this needle reduces the risk of arterial complications. To be able to mani pulate the catheter properly it is essential that the contrast filled tube be clearly visualized with the roentgen-television equipment. It was possible to test many different roentgen television assemblies. Most of these did not permit adequate visualization of the catheter and were therefore unacceptable.

Before inserting the infusion catheters it might be of value to study the vascular anatomy of the pelvis by a routine pelvic ar teriography as related to the gynaecological pelvic examination findings. However since major vascular anomalies are rare, injection of contrast medium into the infusion catheter after insertion in the hypogastric artery in most instances gives enough information as to the appearance of the vascular anatomy and probable distribution of the cytostatic agent.

Since no complications of clinical importance occurred in 40 consecutive catheterizations it seems probable that the technique can be recommended for routine purposes. Moreover there is no need for special and complicated instruments, since ordinary Sel dinger needles, guides and catheters are available in most angiographic laboratories.

For local infusion of cytostatics into the hypogastric artery there is always the problem as to how far the catheter should be introduced into the artery If the tip of the catheter is inserted only about 1 cm there is a risk that the catheter may slip out during the infusion. If on the other hand the tip is introduced too far there is a risk that one or more main branches partially supplying the tumour area will not receive the proper concentration of the cytostatic agent. This is specially true in patients with large pelvic tumour infiltrations from recurrent cervical cancer In the case of choriocarcinoma confined to the uterus it might be of advantage to infuse the agent more or less directly into the uterine artery It should also be emphasized, based on many years of angiographic experience, that a catheter occluding or nearly occluding an artery may give rise to reversal of the direc tion of the blood flow within the artery due to alterations of the pressure gradients. As far as the uterus is concerned there is a

risk that ovarian arteries may take over the principal blood supply

If the catheter is introduced about 3 cm the tip is generally located immediately above the origin of the superior gluteal artery In this position the catheter has no tendency to slip out and the patient can move freely in the bed and even leave the bed for short periods

SUMMARY

Regional chemotherapy by infusion of cytostatics into the hypogastric arteries has been used in certain cases of trophoblastic disease as well as recurrent cervical carcinoma. A method for the percutaneous introduction of thin catheters into these arteries is described. Roentgen equipment needed and technical performance of the procedure is given in detail. A total of 40 catheterizations were carried out and no complications occurred. After some training bilateral catheterization of the hypogastric arteries may be accomplished during 60 minutes or less

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STUDIES ON SERA FROM MEN WITH SPERM ANTIBODIES

BY

BO FJALLBRANT

Introduction

Evidence has been presented that the sperm agglutinating factor in the blood of some men is an antibody. This factor has been connected by some investigators with sterility. In 1954 *Wilson* discovered that serum at high dilution from two sterile men agglutinated spermatozoa. The agglutinating activity of the sera remained after heating at 56° C for 30 min. but was removed by absorption with semen. ABO and Rh blood group differences between sera and spermatozoa did not influence the agglutinating effect. On the basis of these studies Wilson suggested that the sperm agglutination was caused by autoantibodies. *Rümke* (1954) found that one of two male sperm agglutinating sera had sperm immobilizing activity which was increased in the presence of complement. *Cruickshank* and *Stuart Smith* (1959) did not find complement fixing or precipitating antibodies in two sperm agglutinating male sera studied. These investigators showed that the agglutinating activity occurred mainly in the euglobulin fraction and that absorption with AB Rh+ cells did *not diminish* the sperm agglutinating activity. *Rümke* and *Hellinga* (1959) after studying a larger material than the aforementioned stated that the sperm agglutinating factor was an antibody as it was heat resistant, absorbable by packed spermatozoa and detectable in the -globulin fraction after preparative paper electrophoresis. The sperm agglutinins seemed to be specific for spermatozoa and did

not agglutinate erythrocytes leucocytes or thrombocytes. Rumke and Hellinga also stated that the content of complement was normal as were the protein patterns and the rate of sedimentation of erythrocytes in all sera tested. Like Cruickshank and Stuart Smith *Bandhauer* (1966) found no precipitating antibodies in sperm agglutinating sera using seminal plasma Immunofluorescence studies have been performed by *Cruickshank* and *Stuart Smith* (1959) and *Sobbe et al* (1966) who found no γ-globulin fixation on spermatozoa *Feltkamp et al.* (1965) found an interrelation of agglutinating activity of sera and immunofluorescence of spermatozoa in testicular sections and sperm smears exposed to sera. With a fluorescent anti-complement serum they observed complement fixation to spermatozoa

The author has investigated the interrelation of sperm antibodies and sterility in men (*Fjällbrant* 1965 1967 1968 a, b 1969 a b *Fjällbrant* and *Obrant* 1968) The aim of the present studies was to investigate the antibody character of the sperm agglutinating and immobilizing factors in sera from sterile males. Since sperm antibodies in men are looked upon as autoantibodies and since several autoimmune diseases are associated with hyper or dysgammaglobulinemia, the intent was also to investigate the level of immunoglobulins in sera with sperm antibodies.

Material

Blood sera

Men with sperm agglutinins in their blood were selected from a large clinical material of male partners of female patients living in sterile or fertile marriages (*Fjällbrant* 1968 a) Sera from these men—all of which contained sperm antibodies—will be referred to as *patient sera*

Blood samples were also taken from some men in fertile marriages. Sera from these men without detectable sperm antibodies will be referred to as *control sera*

After centrifugation of the blood and decantation of the serum both the patient sera and the control sera were stored at –30 C until analyzed.

Sera from a group of adult blood donors were used as controls

at the quantitation of immunoglobulins. No investigation with regard to the presence of sperm agglutinins was performed. These sera which were stored at +4 C after decantation, will be referred to as *blood donor sera*

Spermatozoa

Semen samples from two donors were used for sperm antibody determinations. When not stated otherwise semen samples from a donor who belonged to blood group B were used for the anti body determinations. The volume of the samples averaged about 6 ml, the sperm density about 140 millions/ml, the percentage of motile spermatozoa about 60 the sperm motility was very rapid. *Spontaneous sperm agglutination was never encountered*, and the ejaculates, in other respects, were also normal.

For some experiments regarding the influence of ABO blood factors, semen samples from a donor belonging to blood group A were used. These samples were of similar quality as those from the B group donor with regard to volume sperm density per centage of motile spermatozoa and motility degree

For the absorption studies a pool of ejaculates from many men was collected for 4 weeks and kept at +4 C. The pool was centrifuged, the seminal plasma decanted and the spermatozoa suspended in phosphate-buffered, isotonic sodium chloride 0 1 M, pH 7 4 and recentrifuged. The supernatant was decanted, the spermatozoa were centrifuged once more and the remaining small supernatant was sucked off from the packed spermatozoa which were used for absorption.

Complement

As source of complement was used pooled fresh guinea pig serum with controlled complement activity (100 % hemolysis at dilution 1 8 in a conventional hemolytic system) Inactivation of complement was performed by incubation at 56 C for 30 min.

Methods

Determination of sperm agglutinin titre was done with Kibrick s macroscopic direct sperm agglutination test (*Kibrick et al.* 1952)

slightly modified as described in an earlier paper (*Fjällbrant* 1968 a) To evaluate the precision of the method as performed by the author the following study was made

Serum from two patients one with low and the other with high sperm antibody level was dispensed in small bottles about 1 ml in each and stored at −30 C. At intervals of 3–7 days one aliquot of each serum was thawed and inactivated. Of each aliquot 3 dilution series were prepared and the sperm agglutinin titre was determined. The procedure was repeated 5 times each time with another semen sample from the same donor Properties of the semen samples and the results of the sperm agglutinin titre determinations are given in Table I

The range was one dilution step at low titre and two dilution steps at high titre The standard deviation was one-half a dilution step at both low and high titres. A variation of sperm density be tween 94 and 196 millions/ml of the semen samples used did not seem to influence the results.

Determination of sperm immobilizing activity was performed as described in an earlier paper (*Fjällbrant* 1968 b) The em ployed measure of immobilizing activity of a serum or serum frac tion was the time required to reduce the percentage of motile spermatozoa from about 70 to 10 in the presence of complement.

Determination of immunoglobulin concentration was done with the single radial immunodiffusion method (*Mancini et al.* 1965) Anti IgG anti IgA and anti IgM sera from goat (Hyland Labora tories Los Angeles Calif) were used.

Gel filtration was performed at +4 C on Sephadex G-200 using a 0 05 M phosphate buffer pH 7 0 with an addition of 0 5 M sodium chloride The columns used were 85×2.5 cm and the flow rate about 8 ml per hour Aliquots of 5 ml serum mixed with 3 % sucrose were added to the column The protein content of the effluent was determined continuously at 254 mμ with an LKB Uvicord I The effluent was collected in 5 ml portions and pooled as indicated in fig 1 the three protein peaks comprising fractions I II and III and the portions between fractions I and II making a fraction called m. The fractions were concentrated about 20-fold by ultrafiltration using collodium tubes (Sartorius Col lodiumfilter) and dialyzed against phosphate-buffered isotonic

Table I. *Sperm Agglutinin Titre of Aliquot of Two Patient Sera Determined with Five Semen Samples from the Same Donor*

Semen					Sperm Agglutinin Titre	
Sample Nr.	Volume (ml)	Density (millions/ml)	Motile Sperms forms (%)	Motility	Patient 26	Patient 37
1	6.0	196	62	very rapid	1 8, 1 8 1 16	1 2048, 1 2048, 1 4096
2	5 6	174	56	very rapid	1 8, 1 8 1 16	1 2048, 1 2048, 1 2048
3	6 1	128	57	very rapid	1 8 1 8 1 8	1 2048 1 2048 1 1024
4	6 0	94	68	very rapid	1 16 1 16, 1 16	1 1024 1 2048 1 2048
5	5 9	95	62	very rapid	1 16 1 8, 1 8	1 4096 1 2048, 1 2048

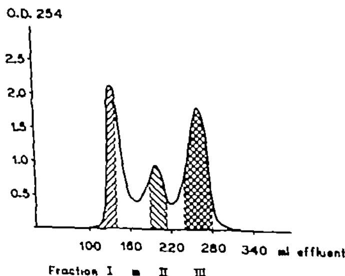


Fig. 1 Gel filtration of serum on Sephadex G-200. Diagrammatic representation of fractions.

sodium chloride pH 7 4 Finally penicillin and streptomycin sul fate were added to a concentration of 3 mg/ml each.

Reduction with mercaptoethanol was performed by adding 0.25 ml 1 M β mercaptoethanol to 1 ml serum. The mixture was kept at room temperature for 4 hours and then alkylation was effected by adding 1 25 ml monoiodoacetamide solution (3 7 mg per ml of isotonic sodium chloride) After 2 more hours at room temperature dialysis was performed for 3 days at +4 C against phosphate buffered isotonic sodium chloride pH 7 4 The mixture was concentrated by ultrafiltration to the original volume of 1 ml in collodium tubes.

Experimental

Absorption of patient sera with spermato oa

Aliquots of 0 5 ml of two inactivated patient sera were mixed with equal amounts of packed seminal spermatozoa from the ejaculate pool incubated at +37 C for 1 hour and at +4 C overnight The mixtures were centrifuged the sera decanted, and the sperm agglutinating and immobilizing activity of the absorbed sera determined. Serum samples from the same men to which no spermatozoa had been added were treated in the same way

The unabsorbed serum samples had sperm agglutinating titres of 1 256 and 1 64 respectively Neither of the sera had any agglutinating activity after absorption The sperm immobilizing activity of the unabsorbed samples was 2 1/2 hours and 4 hours 20 min respectively The immobilizing activity of the absorbed aliquots was 6 3/4 hours and 9 1/2 hours respectively

Gel filtration of patient sera

Fractionation on Sephadex G-200 was performed on 6 patient sera and 4 control sera The obtained fractions were investigated with regard to sperm agglutinins and sperm immobilizing anti bodies. The sperm agglutinin investigation was performed at the dilutions 1 2 1 16 1 128 and 1 1024

The results of the sperm agglutinin titrations are given in table II All fractions of the control sera showed no activity and so did the first fraction from each of half of the patient sera while the others showed a weak agglutinating activity The m fraction from

Table II. Sperm Agglutinating Activity of Fractions of Gel-Filtered Patient and Control Sera. Only Fraction Dilutions 1 2, 1 16 1 128 and 1 1024 Were Tested

Serum No.	Sperm Agglutinating Activity				
	Before Gel Filtration	Fraction I	Fraction m	Fraction II	Fraction III
3	1 128	0	1 2	1 128	0
18	1 256	0	1 16	1 1024	0
30	1 64	1 2	1 2	1 1024	0
31	1 128	1 16	1 16	1 128	0
40	1 128	1 2	1 16	1 128	0
41	1 256	0	1 2	1 128	0
4 controls	0	0	0	0	0

all the patient sera showed low titres (1 2 or 1 16) Fraction II from each of the patient sera had sperm agglutinins at high titres (1 128 or 1 1024) All of the third fractions showed no activity Thus sperm agglutinin activity of the patient sera was found mainly in the second peak corresponding to 7S globulins.

The results as regards sperm immobilizing activity are shown in table III It is noted that the different fractions of the control sera differed considerably with regard to sperm immobilizing activity the highest activity appearing in fraction I and the lowest in fraction III. The activity of fraction I of most of the patient sera was less than that of the control sera In fraction m the activity of most of the patient sera was somewhat higher than that of the control sera. The greatest difference between the patient and the control sera was found in fraction II where the immobilizing activity of the patient sera was very much higher than that of the control sera In fraction III the activity of the patient and control sera was rather equal Thus sperm immobilizing activity of the patient sera was found mainly in the second peak corresponding to 7S globulins.

Heat inactivation and mercaptoethanol reduction of patient sera

Of each of 5 patient sera and 4 control sera one aliquot was *inactivated* at 56 C for 30 min. another aliquot was *reduced with*

sodium chloride pH 7 4 Finally penicillin and streptomycin sul fate were added to a concentration of 3 mg/ml each.

Reduction with mercaptoethanol was performed by adding 0.25 ml 1 M β-mercaptoethanol to 1 ml serum. The mixture was kept at room temperature for 4 hours and then alkylation was effected by adding 1 25 ml monoiodoacetamide solution (3 7 mg per ml of isotonic sodium chloride) After 2 more hours at room tem perature dialysis was performed for 3 days at +4 C against phosphate buffered isotonic sodium chloride pH 7 4 The mixture was concentrated by ultrafiltration to the original volume of 1 ml in collodium tubes

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Table IV *Sperm Immobilizing Activity of Patient and Control Sera after Treatment*

Serum No.	Sperm Immobilizing Activity (in hours)			
	Untreated	Heated 56° C 30 min.	Reduced with Mercaptoethanol	Dialyzed with Saline
18	5 ½	3 ½	8 ½	10
30	8 ½	7 ½	12	16
31	4	4	12	12
40	4 ½	3 ¾	11	11
41	8 ½	6 ½	17	17
4 controls				
mean	47	44	40	44
range	45–48	43–45	39–40	43–44

patient sera were determined after the addition of fresh guinea pig serum and inactivated guinea pig serum. Two dilution series were made up of each serum. In one series 0 1 ml of the 0.3 ml of serum dilution usually used with the method of Kibrick was replaced by fresh guinea pig serum, in the other series by 0 1 ml inactivated guinea pig serum.

The sperm agglutinin titres of the sera were after addition of fresh guinea pig serum 1 128 1 4096 and 1 16 respectively With the addition of inactivated guinea pig serum the titres were 1 256 1 2048 and 1 32, respectively

2. To investigate the influence of complement on the activity of sperm *immobilizing antibodies* the immobilizing activities of 6 inactivated patient sera and one inactivated control serum were determined. Two mixtures were made up of each serum one containing fresh guinea pig serum and the other inactivated guinea pig serum.

The results are given in Table V The immobilizing activity of all patient sera was considerably lower in the absence of complement

Influence of the ABO blood group system on sperm agglutinating and immobilizing activity

1 Serum samples from 17 men selected at random from the sterility clientele and belonging to various ABO blood groups were

Table III. *Sperm Immobilizing Activity of Fractions of Gel-Filtered Patient and Control Sera*

Serum No.	Sperm Immobilizing Activity (in hours)				
	Before Gel Filtration	Fraction I	Fraction m	Fraction II	Fraction III
3	4½	27	14	3½	60
18	5¼	14	17	6½	54
30	8½	20	22	6	58
31	4	23	11	4¼	54
40	4¼	22	12	3¼	54
41	8½	26	13	7	55
4 controls					
mean	47	13	19	26	51
range	45–48	11–14	16–24	23–28	48–54

mercaptoethanol and a third aliquot was *dialyzed* against phosphate-buffered isotonic sodium chloride for 3 days in the same way as the mercaptoethanol reduced aliquots The sperm agglutinin titre of the untreated inactivated and reduced aliquots was determined as was the sperm immobilizing activity of all the aliquots.

The sperm agglutinin titres of the inactivated and reduced aliquots of patient sera were the same or varied within one dilution step. The untreated inactivated and reduced control sera showed no agglutinating activity

As regards sperm immobilizing activity (see Table IV) this was generally somewhat higher in the inactivated than in the untreated aliquots of patient sera The immobilizing activity of the mercaptoethanol reduced aliquots of patient sera was lower than that of the inactivated and untreated aliquots but of the same magnitude as the activity of those aliquots which were only dialyzed. The various aliquots of control sera had about the same sperm immobilizing activity with a tendency to higher activity in the mercaptoethanol reduced aliquots.

Influence of complement

1 To investigate the influence of complement on the activity of sperm *agglutinins* the sperm agglutinin titres of 3 inactivated

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18	5¼	14	17	6½	54
30	8½	20	22	6	58
31	4	23	11	4¼	54
40	4¼	22	12	3¼	54
41	8½	26	13	7	55
4 controls					
mean	47	13	19	26	51
range	45–48	11–14	16–24	23–28	48–54

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Influence of the ABO blood group system on sperm agglutinating and immobilizing activity

1 Serum samples from 17 men selected at random from the sterility clientele and belonging to various ABO blood groups were

Table V *Sperm Immobilizing Activity after Addition of Fresh Guinea Pig Serum as Source of Complement and Addition of Inactivated Guinea Pig Serum*

Serum No	Sperm Immobilizing Activity (in hours)	
	With Complement	Without Complement
37	2 ¼	8 ¼
45	4 ½	23
41	5 ¼	17
35	6 ¼	52
43	22	52
44	36	47
Control	60	60

investigated for sperm agglutinins (at dilution 1 4) with semen both from a donor belonging to blood group A and one belonging to blood group B One serum was sperm agglutinin positive with both semen samples and 16 sera were negative.

2. With semen samples from both the A and B donors the sperm agglutinin titres of 12 patient sera from men in the sterility clientele who belonged to group A B O or AB were determined. The sperm agglutinin titres obtained with the unlike semen samples were the same or showed no significant difference.

3 Aliquots of 5 patient sera 3 from men belonging to blood group A and 2 belonging to blood group O were absorbed with blood group specific substance B until no agglutination of B erythrocytes occurred. The sperm agglutinin titre of one absorbed and one unabsorbed aliquot of each serum was determined with semen from a donor belonging to blood group B who was a secretor of B substance in his semen

The sperm agglutinin titres of the unabsorbed A sera were 1 512 1 256 and 1 256 respectively the titres of the corresponding absorbed sera were 1 512 1 256 and 1 64 The titres of the unabsorbed O sera were 1 8 and 1 16 the titres of the absorbed sera were the same

The sperm immobilizing activity of the unabsorbed and B-substance-absorbed aliquots of two of the aforementioned A sera was

determined with semen from the same B group donor The sperm immobilizing activity of the absorbed and unabsoroed aliquots of each serum was the same 2 h and 5 1/2 h, respectively

4 An aliquot of patient serum from a man belonging to blood group B was absorbed with A substance and A erythrocytes. The sperm agglutinin titre of one absorbed and one unabsorbed aliquot of the serum was determined with semen both from a donor belonging to group A and one belonging to group B.

The sperm agglutinin titre of the unabsorbed serum was 1 1024 with both the A and the B semen the titre of the absorbed serum was 1 1024 with A semen and 1 2048 with B semen.

The immobilizing activity of the absorbed and unabsorbed B serum aliquots was determined with semen from the A donor The sperm immobilizing activity was the same with both aliquots (6 h)

Immunoglobulin levels of patient and blood donor sera

The concentration of IgG IgA and IgM was determined in 40 patient sera and in 40 blood donor sera.

The results, given as mean, standard deviation and range of the concentration of each immunoglobulin within the patient and blood donor groups are indicated in Table VI The men with sperm antibodies had a higher mean level of IgG and IgM but a lower mean level of IgA than the blood donors. Statistical analysis showed that the difference between the groups with regard to the means for IgG was significant ($t = 3.23$ $p < 0.01$) and so was the difference with regard to the means for IgM ($t = 2.65$ $p < 0.01$) The difference with regard to the means for IgA was not significant ($t = -1.32$, $p > 0.05$)

Discussion

Absorption of immune sera by means of antigens is a classical method for depleting sera immunospecifically of antibody activity

The tests were performed at the Municipal Bacteriological Laboratory of Gothenburg, Section of Immunology in collaboration with Dr Lars-Åke Nilsson

Table V *Sperm Immobilizing Activity after Addition of Fresh Guinea Pig Serum as Source of Complement and Addition of Inactivated Guinea Pig Serum*

Serum No.	Sperm Immobilizing Activity (in hours)	
	With Complement	Without Complement
37	2¼	8¼
45	4½	23
41	5¼	17
35	6¼	52
43	22	52
44	36	47
Control	60	60

investigated for sperm agglutinins (at dilution 1 4) with semen both from a donor belonging to blood group A and one belonging to blood group B One serum was sperm agglutinin positive with both semen samples and 16 sera were negative.

2. With semen samples from both the A and B donors the sperm agglutinin titres of 12 patient sera from men in the sterility clientele who belonged to group A B O or AB were determined. The sperm agglutinin titres obtained with the unlike semen samples were the same or showed no significant difference

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The immobilizing activity of the absorbed and unabsorbed B serum aliquots was determined with semen from the A donor The sperm immobilizing activity was the same with both aliquots (6 h)

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The concentration of IgG IgA and IgM was determined in 40 patient sera and in 40 blood donor sera.

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Table VI. *Concentrations (mg/100 ml) of Immunoglobulins i*
Male Patients with Sperm Antibodies and 40 Blood I

	Patients			Blood Donors	
	IgG	IgA	IgM	IgG	IgA
Mean	1830	192	94	1533	222
Standard deviation	495	67	43	302	127
Range	940–3160	27–360	43–250	1050–2450	59–

In the author s absorption experiments with sperm a and immobilizing sera a complete exhaustion of agglu tivity could be obtained by treating the sera with sp The *immobilizing* activity *however was only reduc* same procedure and could not be completely removed l tion with spermatozoa

Since spermatozoa are carriers of A and B blood grou (*Gullbring*, 1957) it could be expected that blood grou tinins might influence the sperm agglutinating activity o Absorption of patient sera with blood group substances however decrease the sperm agglutinating or immobili tivity This finding indicates that the serological activity patient sera as revealed by the agglutination and immobi tests is not caused by antibodies against blood group subs The agglutinating activity of sera was also tested with sp tozoa from semen donors of different blood groups. It was that this use of semen from donors with different blood g did not influence the results of the agglutination test. Thi servation conforms with the aforementioned lack of influenc the ABO blood group agglutinins Statistical analysis of the tribution of ABO blood groups in large sperm agglutinin posi and negative groups have also pointed to a lack of influence the ABO blood group system on the sperm agglutination t (*Fjällbrant* 1968 a and 1969 a)

After gel filtration most of the agglutinating activity was fou in the second peak As this peak is known to contain the 7S ant bodies the finding indicates that the sperm agglutinating anti

bodies are probably of the 7S type. A weak *sperm agglutinating* activity was found in the first peak of some sera, and therefore there may be some agglutinating antibodies with larger molecular size. The results of mercaptoethanol reduction suggest that these antibodies of larger size represent only a minor part of the agglutinating activity of serum. Sulfhydryl compounds such as β-mercaptoethanol dissociate 19S antibodies into smaller units. The unchanged agglutinating activity of sera after reduction with mercaptoethanol *indicates* that the *agglutinating antibodies* are not 19S antibodies and thus aids in characterizing the sperm agglutinating antibodies as 7S antibodies.

The second peak obtained at gel filtration of patient sera showed a sperm *immobilizing activity* which was *markedly* higher than that of the other peaks. Control sera also showed immobilizing activity in the second peak after gel filtration but the activity of the second peak of patient sera was distinctly higher than that of the control sera. These results indicate that the sperm immobilizing antibodies of patient sera are mainly of the 7S type. It is noted, however that in the control sera the sperm immobilizing activity of the three peaks differed between themselves, the highest activity appearing in the first peak and the lowest in the third. This finding points to the existence of other sperm immobilizing factors, an observation which deserves further *investigation* The immobilizing activity was decreased by mercaptoethanol reduction, a procedure that necessitated extensive dialysis before testing of the activity but this decrease cannot explicitly be assumed to be caused by the reduction process, because the decrease of immobilizing activity was of the same magnitude when the sera were only dialyzed.

In the investigations of the incidence of sperm antibodies in groups of men with different fertility *performed* by the author the sera were inactivated at 56 C for 30 min. before their examination for sperm agglutinating and immobilizing activity The studies *reported* on in this paper show that such an *inactivation* can be performed without decrease of the antibody activity in comparison to that of the untreated serum.

The presence of complement did not influence the activity of the agglutinating antibodies but considerably *enhanced* that of

Table VI. *Concentrations (mg/100 ml) of Immunoglobulins in the Sera of 40 Male Patients with Sperm Antibodies and 40 Blood Donors*

	Patients			Blood Donors		
	IgG	IgA	IgM	IgG	IgA	IgM
Mean	1830	192	94	1533	222	72
Standard deviation	495	67	43	302	127	30
Range	940–3160	27–360	43–250	1050–2450	59–545	32–168

In the author s absorption experiments with sperm agglutinating and immobilizing sera a complete exhaustion of agglutinating activity could be obtained by treating the sera with spermatozoa. The immobilizing activity however was only reduced by the same procedure and could not be completely removed by absorption with spermatozoa.

Since spermatozoa are carriers of A and B blood group factors (*Gullbring*, 1957) it could be expected that blood group agglutinins might influence the sperm agglutinating activity of serum. Absorption of patient sera with blood group substances did not, however decrease the sperm agglutinating or immobilizing activity This finding indicates that the serological activity of the patient sera as revealed by the agglutination and immobilization tests is not caused by antibodies against blood group substances. The agglutinating activity of sera was also tested with spermatozoa from semen donors of different blood groups It was noted that this use of semen from donors with different blood groups did not influence the results of the agglutination test. This observation conforms with the aforementioned lack of influence of the ABO blood group agglutinins Statistical analysis of the distribution of ABO blood groups in large sperm agglutinin positive and negative groups have also pointed to a lack of influence of the ABO blood group system on the sperm agglutination test (*Fjällbrant* 1968 a and 1969 a)

After gel filtration most of the agglutinating activity was found in the second peak As this peak is known to contain the 7S antibodies the finding indicates that the sperm agglutinating anti

complement. The levels of IgG IgA and IgM were determined in 40 sera with sperm antibodies and 40 blood donor sera.

Absorption with seminal spermatozoa completely exhausted the sperm agglutinating activity but removed only partially the im mobilizing activity Absorption with blood group substances did not influence the antibody activity of the sera. The sperm agglu tinating antibodies and probably also the immobilizing antibodies were mainly of the 7S type. Incubation at 56 C for 30 min. did not decrease the sperm agglutinating or immobilizing activity Presence of complement enhanced the immunoimmobilization of spermatozoa but did not influence the activity of the agglutinins. *The levels of IgG and IgM were significantly higher in sera from* men with sperm antibodies than in control sera (p<0.01)

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the immobilizing antibodies which conforms with the finding of *RumLe* (1954) This immobilization of spermatozoa mediated by antibody and complement suggests that the process is a cytotoxic one and a parallel to the immobilization phenomenon at e g the TPI test (*Nelson* and *Mayer*, 1949)

Systemic lupus erythematosus is almost invariably accompanied by marked diffuse hypergammaglobulinemia and many other autoimmune diseases e g rheumatoid arthritis, polyarteritis nodosa ulcerative colitis and Hashimoto s thyreoiditis are often associated with moderate hypergammaglobulinemia (*Janeway et al.* 1967) Type 1 dysgammaglobulinemia which involves deficiency of IgA and IgG globulins and increased amounts of IgM globulin can be accompanied by autoimmune processes (*Hin* and *Boyer* 1963) In the present investigation the concentration of immunoglobulins was determined in 40 patient sera and 40 blood donor sera The blood donor sera were not investigated with regard to the presence of sperm agglutinins but as the incidence of sperm agglutinin positivity was 4 8 per cent in male blood donor sera (Fjällbrant 1968 d) this circumstance can not be expected to diminish the value of the blood donor sera as controls to any significant degree The immunoglobulin levels in the blood donors were within the usually indicated range of normal values with the exception of the mean of IgG concentration which was slightly higher than usually found. The mean concentrations of IgG and IgM in the sera from patients with sperm antibodies were statistically significantly higher than the means of the blood donor sera This observation of raised immunoglobulin levels in sera with sperm antibodies suggests that formation of measurable sperm antibody levels in men is associated with a generally increased production of antibodies

SUMMARY

Male sera with sperm agglutinating and immobilizing activity were studied by absorption with seminal spermatozoa and with A and B blood group substances by filtration on Sephadex G-200 reduction with mercaptoethanol and incubation at 56 C for 30 min The activity was also determined in the presence and absence of

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From the Institute of Zoophysiology (Professor P E Lindahl) Uppsala Sweden

CYCLICAL VARIATIONS IN THE ARBORACEOUS CRYSTAL PATTERN OF AIR-DRIED SEMINAL FLUID

BY

J. E. KIHLSTRÖM AND D. FJELLSTRÖM

For a long time it has been looked upon as a fact that while the female sexual functions are cyclical in nature the male sexual functions are not (*Bullough* 1951) This conception seems to be supported by the studies of the sexual differentiation of the brain (*Harris* 1964) However the existence in both sexes of similar feed-back mechanisms of interacting gonadotrophic and gonadal hormones makes it plausible to expect the occurrence of cyclic phenomena, corresponding to the oestrous cycle in the male also. Such rhythmic changes were first observed in rabbits (*Doggett* 1956 *Kihlström* 1958) and have since been demonstrated to occur in cattle (*Kihlström*, 1962) rats (*Kihlström* 1965) mice (*Kihlström* and *Järnebrand* 1968) and possibly also in man (*Doggett* and *Kellers* 1962 *Hornstein* 1965 *Månsson* 1965) In all species so far studied the duration of this male cycle is approximately the same as that of the corresponding female sexual cycle

The most important parameters shown to vary cyclically are the olume of ejaculate (*Kihlst öm* 1958 *Kihlst öm* 1963 *Kihlstrom* and *Hultnas* 1968 *Degerman* and *Kihlström* 1961) the number f sperm cells (*Doggett* 1956 *Doggett* and *Kellers* 1962 *Kihlström* 1963 *Kihlström* and *Hultnas* 1968) the sexual drive (*Degerman* and *Kihlström* 1961) the fertility (*Kihlström* 1962, 1963 *Kihlstrom* and *Hultnas* 1968) the exfoliation of cells from the urethra (*Kihlst öm* and *Hornstein* 1964 *Hornstein* 1965 *Kihlst öm* and *Järnebrand* 1968) the spontaneous ejaculations

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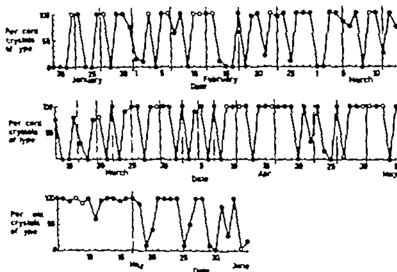


Fig. 1. Daily variation during six months of the percentage of crystals of type R in the representative area of the preparations. Dotted lines mark the mid-points of the periods during which crystals of type R occur in the preparations.

temperature of the room was thermostatically regulated, and the food was always given at the same time of the day. Semen was collected once a day between 1 and 2 p.m. and delivered into a graduated test tube, used in determining the volume of the ejaculates.

The ejaculates were immediately freed from spermatozoa by centrifuging for 10 minutes at 975 g. A small drop of the seminal fluid was placed on a well cleaned object glass-slide and dried in air at a temperature of 21–23° C. The preparations obtained in this way were permanent and easy to study under the microscope.

As the decreasing thickness at the periphery of the drops may influence the forms of the crystals only a central area of each preparation, defined in the following way, was studied. By using a microprojector an image (magnification 120 ×) of the dried drop was drawn on millimeter-squared paper. A circle, as large as possible, was then inscribed in the drawing of the dried drop.

(*Kihlström* 1965) and the body temperature (*Degerman* and *Kihlström* 1964) Some experiments also indicate a hormonal regulation of these cyclic changes (*Hornstein Kihlström* and *Degerman* 1964 *Inoué* 1965 a and b *Exley* and *Corker* 1965) A review of the literature is given by *Kihlstrom* (1966)

In the females the characteristic palm leaf like crystals appearing in dried samples of cervical mucus, first observed by *Papanicolaou* (1933) occur abundantly around the day of ovulation, but are uncommon or even completely missing during other parts of the menstrual cycle (*Rydberg*, 1948) As early as 1921 similar crystals were observed in semen from cattle horse and rabbit (*Yamane* 1921) Nevertheless the occurrence of such crystals in semen has later been denied (*Rydberg*, 1948 *Rodrique Villa* 1958) Similar crystals also appear in the secretions from the prostate (*Rodrique. Villa* 1956 1958 *Vartapetov* and *Demtjenko* 1965) and the urethral glands (*Belonoschkin* 1964) Arboraceous crystals in semen, if they exist, could possibly be expected to be subject to cyclic changes such as those seen in the cervical mucus. Moreover the characteristics of the crystals are possibly less dependent upon extraneous factors than are the different expressions of the sexual activity for example the volume of ejaculate earlier used in studying the male sexual cycle For these reasons we tried to confirm *Yamane* s observations in order to follow the daily variation of crystallization in samples of semen.

Material and Methods

Semen was collected from 12 rabbits (Swedish Country Breed) and from 21 bulls (Swedish Red Breed) by means of artificial vaginas and from two men by masturbation.

One of the rabbits was used for a continuous study of the crystals in dried seminal fluid from ejaculates yielded once a day from the middle of January to the beginning of June For this particular buck the mean duration of the male sexual cycle was known from earlier studies to be between 6 and 7 days (*Degerman* and *Kihlström* 1968) The animal was kept in a separate cage in a windowless room and subjected to an artificial cycle of daylight with a dark period extending from 8 a.m. to 8 p.m. The



Fig. 2 Crystals of type R. Magnification 1030 ×

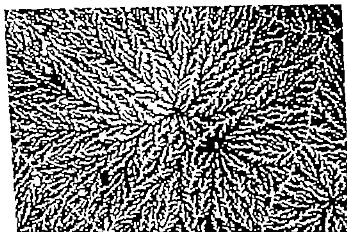


Fig. 3 Crystals of type A. Magnification 1030 ×

are perpendicular to the stems. The branches as well as the stems are practically straight. *In a very few cases only the stems diverge* from a common centre. The second pattern of crystals (type A, acute-angled crystals, Fig. 3) has branches forming acute angles

Another circle with the same centre and a radius half that of the large one was taken as the representative area. Within this area the zones containing different types of crystals were marked on the paper and calculated as percentages of the total surface with an accuracy of ± 2.5 per cent The figures obtained in this way were arranged chronologically with a 24-hour period as a constant interval of time A series thus obtained is diagrammatically represented in Fig 1 The method of *Kermack* and *McKendrick* (1937) was used to investigate whether the different crystallization patterns appear at random or not. More detailed information about a possibly occurring cyclic variation was obtained by applying a *serial* χ^2 test, related to the method of serial correlation, as follows Within the chronological series mentioned above the data were classified into two categories, *for example* one representing preparations with more than 50 per cent crystals of type R (see below) and the other representing preparations with less than this figure The series was then displaced along its own copy 1 2, 3 etc time units (one time unit = 24 hours) After every displacement the number of data in the undisplaced series coinciding with data of the same category in the displaced series were noted. The number of coinciding pairs thus obtained was then compared by means of the χ^2 test with the corresponding number calculated for a random distribution of the data of the two classes. A cyclic variation if existing, will manifest itself as a higher number of coinciding pairs than expected This will happen when the length of the displacement is close to that of the cycle or to a multiple of that length.

Results

After eliminating spermatozoa and other particles from the ejaculates palm leaf like crystals are seen in about 50 per cent of the samples from bulls and men and in the overwhelming majority of the samples from rabbits. For various reasons a more detailed study was restricted to rabbit semen

In air-dried preparations of seminal fluid from rabbits two distinctly different types of ramified crystals were observed. In the first type (type R, rightangled crystals Fig 2) the branches

with the stems. The branches as well as the stems are gently undulating and a number of stems diverge from a common point in the centre of each crystal. When the crystals of this type occur abundantly and close together they form a polygonal pattern (Fig. 4) In addition crystals having a form transitional between those of type R and type A (Fig 5) may be seen.

As seen from the photomicrographs in Fig. 6 and the diagram in Fig. 1 the form of the crystals in seminal plasma from the rabbit studied serially varied from day to day in a more or less regular way Most of the preparations were dominated by crystals of type R, sometimes intermingled with the transitional form of crystals. The occurrence of the crystals of type A was more restricted, and the polygonal pattern appeared sparsely and rarely dominated the preparations. As seen from Table I the distribution of these different patterns of crystallization is far from random, which strongly indicates the existence of a cyclic variation.

The results arrived at when applying the serial χ^2-test described above are given in Table II As seen from the table the occurrence of preparations with crystals of type R as the predominant type shows a statistically significant rhythmicity with a length of seven days. The third multiple of this length also gives a statistically significant value. Preparations containing exclusively crystals of type R give statistically significant values, when the length of the displacement is a multiple of seven. We are thus compelled to conclude that the crystallization pattern in seminal fluid from rabbits varies cyclically and that the length of this cycle is very close to the mean length of the male sexual cycle in this species (*Kihlström* 1966) as well as to the known length of the male sexual cycle of this particular individual (*Degerman* and *Kihlström* 1968)

Furthermore there is a statistical relationship between the seminal volumes which are known to vary cyclically (*Kihlström* 1966) and the crystallization pattern. Thus, volumes obtained on the first day during periods with exclusively crystals of type R are significantly smaller than the volumes obtained on the most central day during such periods (mean values 0 46 ml and 0 56 ml respectively $t = 2.44$ $f = 44$ $p < 0.01$) Volumes obtained on

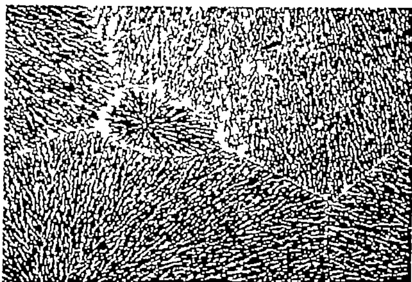


Fig 4 Crystals of type A arranged in a polygonal pattern
Magnification 1030 ×



Fig 5 Crystals transitional between type R and A
Magnification 1030 ×

Table I. The Observed Distributions of the Intervals Between Days with Different Types of Crystals Compared with the Theoretical Distribution t be Found in a Series of Random Numbers

Crystal Pattern	Intervals in Days	Number of Intervals Observed		Number of Intervals to be Found in the Theoretical Distribution	
Crystals of type A	3	10		14.0	$\chi^2 = 6.59$
	4	9		11.7	$p < 0.025$
	5	7			Mean length:
	6	5			4.7
	7	0	16	9.3	
	8	3			
	11	1			
The polygonal pattern of crystals of type A	3	3		11.2	$\chi^2 = 33.22$
	4	4		9.3	$p < 0.0005$
	5	7			Mean length.
	6	7			5.8 days
	7	3	21	7.5	
	8	3			
	16	1			
Crystals of type R	3	5		9.2	χ^2 13.93
	4	4		7.7	$p < 0.0005$
	5	2			Mean length:
	6	4			6.6
	7	1	14	6.1	
	8	5			
	13	1			
	27	1			

the first day following upon such periods are also smaller than those obtained on the most central day (mean values 0.49 ml and 0.56 ml respectively $t = 1.80$ $f = 57$ $p < 0.05$) The conclusion must be that the seminal volume increases up to a maximum and begins to decrease again during periods with exclusively crystals of type R. Moreover minimum values of the seminal volumes occur more often than theoretically expected 24 hours after a maximum of the crystals of type A ($\chi^2 = 5.52$ $p < 0.025$)

These facts strongly indicate that the cyclic variation in the crystal pattern of the dried seminal fluid is a further expression of the male sexual cycle previously observed (*Kihlström* 1966)



Fig 6 Microphotographs showing crystallization patterns during five successive days (February 25th to March 1st) Magnification 1030 ×

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STUDIES ON THE HUMAN PLACENTA

III. Vascularization of the Young Foetal Placenta

A. Vascularization of the Chorionic Villus

BY

FINN BØE

Introduction

In the preceding study (*Bøe* 1968) three types of structures were described

1. Oblong structures of uniform diameter or slightly swollen, studded with slim projections. These structures constitute the majority of the villi, i.e. the main type.
2. Markedly swollen, translucent (oedematous) villi with smooth surface, i.e. without projections.
3. Thin, threadlike syncytial bands, likewise, without projections.

Numerous transitional forms occur between the first (main) type and the two remaining secondary types.

The present paper gives a description of the vascularization of these anatomical structures, the relationship of the foetal vasculature to the cell islands and the basal plate and the vascularization of the terminal ramifications of the chorionic tree i.e. of the terminal branches from which the majority of villi arise.

Because different problems are involved, it was found convenient to describe and discuss the material available in two different parts

A. Vascularization of the chorionic villus.
B. Vascularization of the translucent villus and the syncytial band, with special reference to the cell islands and the basal plate.

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B. Vascularization of the translucent villus and the syncytial band, with special reference to the cell islands and the basal plate.

Material and technique in the present study were similar to those described previously Fresh placentae, removed by hysterotomy were placed in tepid saline solution. The umbilical cord was cut about 3 cm from its insertion and blood forced out by repeated careful pressure. Indian Ink was used as contrast medium and injected into the umbilical artery In some cases retrograde filling of the vascular system was carried out by injection of contrast medium into the umbilical vein.—The injection should be carried out slowly and at low pressure because of the high fragility of the foetal capillaries. Even a moderate elevation of the intra-capillary pressure proves sufficient to provoke rupture of the capillary walls with the formation of extravasates in the villous stroma.—The dissection method and the treatment of the specimens selected for study were described in the two preceding papers (*Boe* 1967 1968)

Results

1 *Vascularization of the Minor Chorionic Structures (Syncytial Buds and Projections)*

The paravascular net (PVN *Boe* 1953) is a system of anastomosing vessels of capillary size which constitutes a system of arterio-venous communications (shunts) between the afferent arteries and the efferent veins of the terminal branches and the villi The PVN appears mainly and constantly in the terminal branches and the villi of the main type (Figs. 1 and 2)

The net is sited characteristically subjacent to the trophoblastic epithelium lining the latter like a tapestry (Figs. 3 and 4) Therefore, the net might equally well be termed the subepithelial (subtrophoblastic) net and is a two-dimensional structure. Anastomosing capillaries do not appear in the remaining stroma

In the mature placenta the minor chorionic structures (buds and projections) are vascularized from the PVN exclusively (*Boe* 1953) In the young placenta, likewise the minor chorionic structures are vascularized from the PVN (Figs 1 and 2) However in these structures separate vessels are not demon-

My original suggestion was th paravascular capillary network

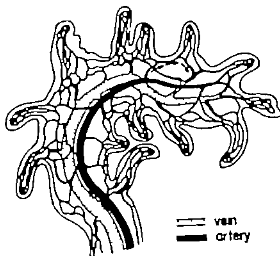


Fig. 1. Vascularization of chorionic villus. Schematic drawing. The projections are vascularized from the paravascular net. Arrows indicate shunt mechanism.



Fig. 2. Chorionic villus, main vessels and PVN well filled with contrast medium. Short projections (above) vascularized from the PVN. 12 weeks. Transillumination. × 170

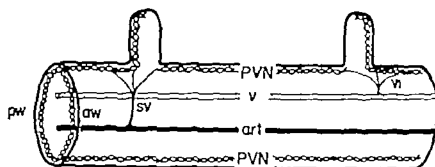


Fig 3. Longitudinal section of a chorionic villus. Schematic drawing. One projection vascularized from the paravascular net (PVN)

art: artery
v: vein
aw: anterior wall
pw: posterior wall
sv: supply vessels
vi: venous inlet



Fig 4 Cut surface of a chorionic villus PVN in the anterior wall in focus (below) appearing blurred in the posterior wall (above) One main vessel level with the PVN (above right) Remaining stroma depleted of capillaries 9 weeks Transillumination ×500

strable, their supply vessels being indistinguishable in size from the capillaries lining the epithelium For that reason the PVN will give the impression of continuing unbrokenly into these structures, whose remaining stroma appears devoid of vessels. Like the PVN the position of these capillaries is subepithelial

Since 1953 the PVN has been described by *Mayer et al.* (1956) *Crawford* (the pre-villous plexus 1961) *Arts* (1961) *Alvarez* (1964) *Aladjem* (1967) and by *Cibils* (1967)

The *supply vessels* of the PVN arise from the main vessels of the terminal branches and the villi (Figs. 1 and 3) The supply vessels are of pre-capillary or capillary size. The vessels usually ramify their branches following a diverging course until they split up in the PVN The arterial (afferent) branches supplying the PVN and the venous (efferent) branches draining the net, exhibit a very similar pattern in the young placenta. The vessels arise at irregular intervals and in every plane, apparently independent of the position of the minor chorionic structures which are vascularized from the PVN The venous inlets seem to be more numerous than are the arterial supply vessels.—Anastomoses were not observed in the supply vessels.

2. *Vascularization of the Terminal Ramifications*

The *minor chorionic structures* are vascularized from the PVN Their supply vessels are indistinguishable in size from the vessels of the net

A *villus* may be defined as a chorionic structure with separate supply vessels. These vessels are distinguishable from the vessels of the PVN and the capillaries by their larger size.

A *terminal branch* may be defined as a ramification of the chorionic tree from which villi arise. The separate vessels of the villus arise from the main vessels of the terminal branch.

The superficial PVN in the terminal branches communicates with the PVN in the villi, the latter continuing unbrokenly into the capillaries of their buds and projections. Obviously the PVN and the capillaries constitute an anastomosing continuum, an anatomical entity A villus may simply be regarded as a big projection, with additional separate supply vessels. The capillaries of the projections may be regarded an extension of the PVN

The numerous communications (syncytial bands) between the villi were never entirely vascularized. The PVN thus, represents the only vascular connection between the villi. The chorionic villus, therefore must be regarded as a vasculatory unit (*Bøe*

1953) In this view *Crawford* (1956 a, b) *Arts* (1961) and *Boyd* and *Hamilton* (1967) agree.

The main vessels There are two vessels in every villus and terminal branch, one artery and one vein. The course of the vessels shows a somewhat different pattern in the different villi and branches. The two vessels may run fairly parallel though separated more than in the big vessel-conveying stems. The artery usually follows a more superficial course than does the vein, and the vessels may cross —Very frequently the vessels show a diverging course gradually approaching the PVN artery and vein diametrically opposed until the vessels terminally split up in the apical part of the PVN —In other specimens the vessels may run nearly their entire course superficially close beneath the PVN (Fig 4) —Finally and not infrequently the vessels run in the same plan as the PVN In the latter specimens the remaining stroma appears completely empty of vessels Tortuosity of vessels was not observed.

It appears from the preceding description, that the vascularization of the chorionic structures is less complicated in the young placenta than it is in the mature organ. The different pattern of arteries and veins so characteristic of placental maturity (*Boe* 1953) could hardly be traced in the young placenta.—The sinusoidal expansions of the capillaries could not be demonstrated in the capillaries of the young placenta.

Discussion

The PVN and the capillaries may be regarded as an anatomical entity the capillaries of the projections being an extension of the PVN The projections greatly increase the surface of the syncytium, and, accordingly facilitate the exchange of metabolites between the two circulations

The anatomical structure of the PVN is characteristic of a shunt mechanism allowing blood to flow directly from the arterial to the venous side of the system eventually by-passing the projections. In this way the PVN will act as a buffer system for the regulation of the haemodynamics in the villi. This mechanism may seem necessary because the chorionic villus must be re-

garded a circulatory unit, the PVN constituting the only vascular link between the projections (and between the villi)

Like the PVN the anatomical position of the capillaries is subepithelial, two-dimensional, lining the inside of the syncytium like a tapestry as close as possible to the maternal blood in the IVS This is in accord with the function of the capillaries. Presumably the entire capillary system (the PVN included) is concerned solely with the exchange of gases and metabolites between the maternal and the foetal circulations. Accordingly the emaining stroma appears devoid of anastomosing capillaries.

Presumably a gradual fall in the blood pressure in the entire vascular system, seems necessary to circulation in the structures.

The evidence presented in this study may be explained by considering the chorionic tree as a structure in continual growth. Consequently sharp distinction between the different chorionic structures does not appear

SUMMARY

1 The terminal branches, from which the majority of villi arise represent the terminal ramifications of the chorionic tree.

2. The main (stem) vessels of the terminal branches supply the villi with their separate vessels.

3 The paravascular net is a system of anastomosing vessels of capillary size, interposed between the artery and the vein (main vessels) in the terminal branches and the villi. The latter vessels supply the net with branches of pre-capillary or capillary size.

4 The paravascular net continues unbrokenly into the anastomosing capillaries of the syncytial buds and projections. Together the two vascular systems form a continuum, an anatomical entity of which the capillaries of the projections are an extension.

5 The anatomical position of this continuum is subepithelial closely beneath and adjacent to the trophoblastic epithelium. Thus, the structure is essentially two-dimensional.

6 The chorionic villus is considered as a circulatory unit. The paravascular net represents the only vascular communication between the projections and between the villi.

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STUDIES ON THE HUMAN PLACENTA

III. Vascularization of the Young Foetal Placenta

B. Vascularization of the Translucent Villus and the Syncytial Band, with Special Reference to the Cell Islands and the Basal Plate

BY

FINN BØE

1. *The Translucent Villus*

The extreme type, which is of cucumber form with a smooth surface is an avascular structure, i.e. the vascular system apparently has disintegrated. The different stages in this retrogressive process may be observed in the transitional forms, from the fully vascularized main type studded with slim projections, to the avascular type without projections. The latter type and the transitional forms occur everywhere in the intervillous space (IVS). The majority however are in close relationship to the cell islands and to the basal plate.

In this retrogressive process two features are almost constant,

1. the process starts in the apical part of the villus and the terminal branch,
2. the system of capillaries i.e. the paravascular net (PVN) and the capillaries of the projections, disintegrate before the main vessels do.

a. *The system of capillaries*

In several well vascularized villi, swollen, bulb-shaped, translucent projections appear scattered between slim projections (Bøe 1968). The swollen villi show a predominance of bulb-shaped

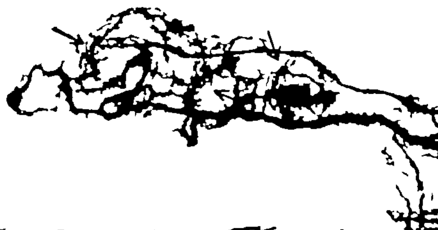


Fig 1 Villus with plump projections Three lateral projections (below) arrows indicate the border of another three more centrally placed projections.—Coarse and fine vascular pattern. Areas of projections non-vascularized. (Two photographs taken at slightly different levels are put together) 9 weeks. Transillumination. ×84

projections (Fig 1) with short, expanded capillaries connected by tiny filaments. The capillaries are irregularly distributed at the base or over the surface of the projections. Areas of the surfaces are devoid of vessels to a varying degree (Fig 1) —When projections are close together apical expansions may assume a pattern resembling of a garland.

On the surface of the swollen villus very tiny projections of varying length are observed. These microprojections may be of uniform diameter or they may terminate like a knot forming a drumstick like structure In a third variety the upper part of a bulb-shaped projection appears constricted into a tiny protrusion. Invariably these structures are avascular or very poorly vascularized (Fig 2)

In the swollen villi with plump (wartlike) projections similar changes are observed in the PVN (Fig 3) —In advanced changes the capillary system having assumed a uniformly tiny filiform appearance.—When the retrogression is still more advanced, only remnants of the system appear forming scattered, tiny filaments.



Fig. 2. Swollen villi with microprojections drumstick-shaped one (lower arrow) and one tiny protrusion protruding from the top of plump pr jec tion (upper arrow). Several other fine projections are seen. Above, extreme left avascular smooth villus from neighbouring branch. 9 weeks. Dark f eld. ×66

—In the extreme type of translucent villus the entire capillary system has disintegrated (Fig. 4 A B C)

When contrast medium is injected into the arterial system and appears in the venous system, the medium, obviously must have passed *via* the capillary system (*vice versa* if the medium was injected into the venous system) The preservation of capillaries, therefore, is a necessary condition for the filling of the opposite vessel with medium. Even when the major part of the capillary system has disintegrated, intact vessels in a limited area prove sufficient to allow passage of contrast medium into the opposite vessel (Figs. 3 and 7) The passage of medium occurs even with advanced, uniform capillary retrogression, *i.e.* in the stage of filiform appearance. However when disintegration has proceeded to a stage where the capillaries are no longer communicating,

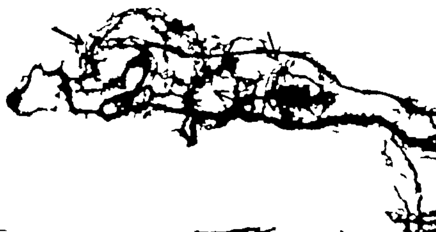


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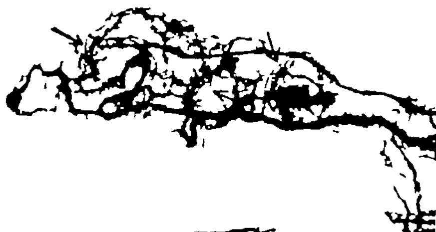


Fig. 1 Villus with plump projections. Three lateral projections (below) arrows indicate the border of another three more centrally placed projections.—Coarse and fine vascular pattern. Areas of projections non-vascularized. (Two photographs taken at slightly different levels are put together) 9 weeks Transillumination. ×84

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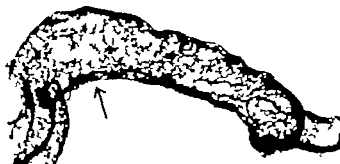


Fig. 4 A. Markedly swollen villus with smooth surface. Artery can be followed shadowlike beyond the limit of contrast medium (arrow), terminating in U-formed configuration in the apical part (right). A few filaments visible on the surface. Vein not visible. 10 weeks. Transillumination. ×66.



Fig. 4 B. Markedly swollen villus with smooth surface. Part of the artery appears shadowlike. Capillaries and vein not visible. 10 weeks. Transillumination. 105

projections the supply vessels from the PVN being indistinguishable in size from the capillaries. In moderate retrogression, i.e. in incipient disintegration of the vascular system, the pattern becomes more apparent than in the fully vascularized structure. The supply vessel arising from the main artery ramifies, giving off branches to the PVN and terminating in vessels supplying the projections (Fig. 5 A). One vessel enters a projection adjacent to the epithelium, forms an apical loop and continues on



Fig 3 Markedly swollen villus with plump projections Venous filling. Artery (above) fades away in the neck (arrow) the vessel filled from the vein (below) *via* the capillaries Coarse and fine pattern of the capillary system. 13 weeks. Transillumination. ×105

passage of medium naturally becomes impossible and no medium can be seen in the opposite vessel (Fig 4 A B C)

The filling of the opposite vessel *via* the capillary system is limited to the region adjacent to the capillary area through which the contrast medium has passed. The rest of the vessel up to its origin from the main vessel is empty of medium (Figs. 3 and 7) —This common finding seems to exclude retrograde filling, and, likewise filling through *arterio-venous anastomoses*, which, apart from the PVN are generally supposed to occur very infrequently in the placenta.

b *The main vessels and their branches*

In the well vascularized villus the PVN gives the impression of continuing unbroken into the anastomosing capillaries of the

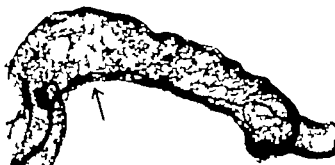


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Fig 5 Swollen villus, moderate regressive changes. *A* Arterial pattern Supply vessel from the main artery (upper arrow) gives off branches to the PVN and terminates in three capillary vessels, one to each of three projections (lower arrows) 12 weeks T emulbimination ×84

mately fade away shadowlike in apical direction. Probably the reason for the medium failing to penetrate the vessel or penetrating imperfectly giving a shadow of the vessel, is occlusion f the lumen by endothelial hyperplasia, which is a frequent finding in the histological picture Avascular villi were recently described by *Bâdă* ail *et al.* (1967)

The small and medium sized stems attached to the basal plate



Fig. 4 C Markedly swollen villus with smooth surface. Artery can be followed shadowlike beyond the limit of contrast medium (arrow) Capillaries and vein not visible. 10 weeks. Transillumination. ×105

the opposite side as the efferent (venous) capillary The latter vessel receives tributaries from the PVN draining the net, and joins the main vein (Fig 5 B)

In markedly swollen villi with plump projections the supply vessels show alternate tiny (contracted) and expanded (dilated) areas (Fig 6) The main vessels also may appear dilated. Frequently the expansions are most conspicuous in the apical part of the villus (Figs 1 and 10) —In more advanced cases the dilated capillaries apparently coalesced into a single vessel, uniting the afferent and efferent vessels to form a loop (Fig. 8 A B) When this vascular loop is demonstrable the capillaries have disintegrated, or only remnants are visible Therefore it is more likely that the expansions are formed by coalescence of capil laries than by dilatation of pre-existing vessels.—In terminal branches as well the vessels may terminate in loop formation (Fig 8 A)

The main vessels most frequently terminate blindly in the swollen, translucent apical area, the contrast medium fading away The more the disintegration has proceeded *i.e* the nearer the formation of the extreme type the shorter will the vessels appear —In many specimens the vessels may be clearly visible for some distance beyond the limit of medium, and they ulti

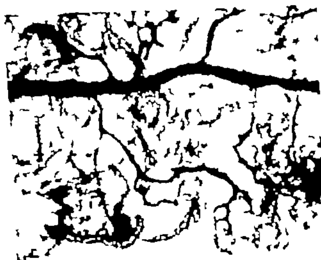


Fig 6 Terminal branch. Main artery on level with the PVN. Tiny supply vessels from the artery show expansions. Coarse (expanded) and fine capillaries in the plump flattened projections areas of which appear non-vascular aged 10 weeks. Transillumination. ×105

area. More rarely the artery may run a short distance into the area giving off no branches.—Not infrequently the vessels show a terminal chandelier pattern, bending into the IVS from the border of the translucent area (Fig. 9)

The characteristic swelling of the translucent villus apparently is due to oedema in the villous stroma. The genesis of this oedema is not obvious from the results of the present study No signs of stasis were apparent. Increased permeability of the capillaries during their disintegration, may be another possibility

2. *The Syncytial Band*

The syncytial bands, forming a framework in the IVS most frequently are avascular structures. Nevertheless, the bands may sometimes appear to be vascularized, but hardly ever in their



Fig 5 B Venous pattern (photograph taken from the back of the slide) Tributaries to the main vein (arrows) drain the three projections and receive tributaries from the PVN 12 weeks T ansillumination. ×84

(anchoring villi) usually are moderately swollen and may appear well vascularized. At their attachment, the structure is moderately swollen and translucent, without projections. When these anchoring villi are detached from the basal plate their apices are covered by a cap of opaque tissue severed from the plate (Fig. 9) —Usually the main vessels fade away or ter minate more abruptly on the border of the translucent apical



Fig. 6 Terminal branch. Main artery on level with the PVN. Tiny supply vessels from the artery show expansions. Coarse (expanded) and fine capillaries in the plump flattened projections, areas of which appear non-vascular aged 10 weeks Transillumination. ×105

area. More rarely the artery may run a short distance into the area, giving off no branches.—Not infrequently the vessels show a terminal chandelier pattern, bending into the IVS from the border of the translucent area (Fig. 9)

The characteristic swelling of the translucent villus apparently is due to oedema in the villous stroma. The genesis of this oedema is not obvious from the results of the present study No signs of stasis were apparent. Increased permeability of the capillaries during their disintegration, may be another possibility

2. *The Syncytial Band*

The syncytial bands forming a framework in the IVS most frequently are avascular structures. Nevertheless, the bands may sometimes appear to be vascularized, but hardly ever in their



Fig 7 Small stem. Venous filling Left: Swollen villus with smooth surface. Vein fades away shadowlike in the apical area. PVN preserved in a limited area in the neck Contrast medium appears in a fragment of the artery (left arrow) filled *via* capillaries.—Right: Complex of villi. Main vein fades away inlet of a supply vessel (centre arrow) Filiform appearance of the capillary system. Right arrow: Medium in the main artery having passed *via* the capillaries, terminates abruptly A supply vessel arises from the artery 13 weeks Dark field ×33.

entirety The bands thus do not form vascular communications between the villi

Usually the band is most extensive at its origin most frequently from the apical part of the villus gradually narrowing during its further course into thin smooth threadlike and avascular structures

The syncytial band is vascularized from the main vessels of the villus from which it arises. In some specimens both vessels may be followed for some distance united by remnants of the PVN until fading away Not infrequently the vessels terminate in loop formation. Perhaps most frequently only one vessel appears filled with contrast medium, the PVN and the opposite vessel being absent. Occasionally PVN from the villus may

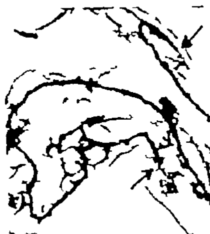


Fig. 8. A. Venous filling. Below: Vascular loop in smooth, swollen terminal branch. Lower arrow: Reflux of contrast medium into the artery terminates; the vessel can be followed shadowlike beyond this point.—Above: Vascular loop in smooth, swollen villus. Upper arrow: Reflux of contrast medium into the artery terminates, the vessel fades away. 9 weeks. Transillumination. 84



Fig. 8. B. Vascular loop in a translucent villus. 9 weeks. Transillumination. 84



Fig. 9 Villous stem ("anchoring villus") disrupted from the basal plate. Chandelier pattern of the vessels bending into the IVS from the apical translucent area. Opaque tissue (top left) severed from the basal plate. 9 weeks Transillumination. ×53

continue for some distance into the band and then fade away — If the short projections are vascularized, they are supplied from the PVN — In each specimen remnants of capillaries may be found near the origin of the band

3 Relationship of the Vasculature to the Cell Islands

The cell islands are avascular structures although vascularized foetal structures frequently occur in close relationship to the surface of the islands.

Terminal branches and even major structures which are filled to a varying degree with contrast medium may be attached to the cell islands by their free, apical part very similar to the attachment of the anchoring villi to the basal plate. Likewise vascularized villi and projections may be observed attached to



Fig. 10. Small villus (above right) attached to cell island, capillaries form tiny loops level with the surface – Vascular loop (expanded) and remnants of capillaries in translucent villus (left) ttached to the island by a short, avascular syncytial band. 9 weeks. Dark field. ×84.



Fig 11 Translucent villus attached to cell island on lateral aspect. Vascular expansions (garland pattern) in the main vessel adjacent to the surface. Ordinary calibre and course of the opposite vessel (below), fading aw y in apical direction (centre right) 9 weeks. T ansillumination 84

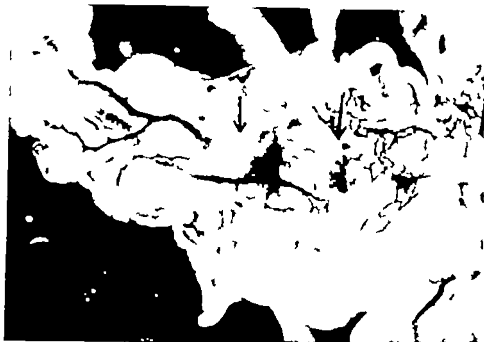


Fig. 12. Translucent villus attached to a cell island on lateral aspect. The apical part (extreme left) is vascularized *via* main vessels, intimately adjacent to the surface. Note the poor filling of these vessels especially the upper one (arrows). 9 weeks. Transillumination. ×84

the islands by their free apical part (Fig. 10) and structures may also appear attached to the islands on their lateral aspect (Figs. 11 and 12).

The degree of vascularization varies considerably. Close to the surface the dilated vessels (garland pattern) predominate (Figs. 11 and 13 A). Level with the surface the adjacent wall of the attached structure apparently disintegrates. The dilated vessels seem to grow out of the structure exhibiting a short radial course. They attach clawlike to the surface (Fig. 13 B).—In other specimens the vessels show a more elongated course thus *forming a small corona* (Fig. 13 C), *the vessels fading away on* the surface.—When the structures are attached on their lateral aspect the main vessel adjacent to the surface may exhibit definite expansions (garland pattern), whereas the opposite vessel, being free of the surface, shows a more normal calibre and

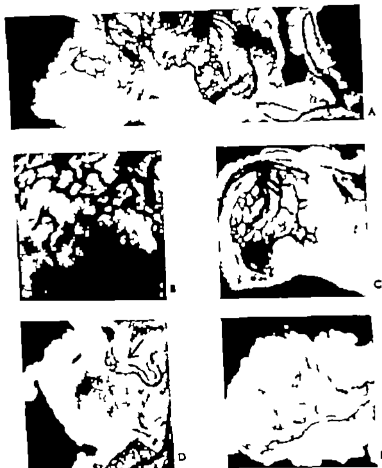


Fig. 13 A: Vascularized structures attached to two cell islands. Right island: Expanded capillaries (garland pattern). Left island: Widemeshed tiny capillaries level with the surface.—Extreme right: Vascular loop in villus attached to the island. 9 weeks. Dark field. 84. B: Expanded capillaries attach clawlike to the surface of cell island. 9 weeks. Dark field. ×105. C: Small villus attached to cell island. Capillaries grow out of the structure, form small corona on the surface. 9 weeks. Dark field. ×84. D: Small villus attached to the surface of cell island, afferent and efferent vessels visible. Note the poor vascularization of the vessels level with the surface (arrow) and the richer filling of the projections protruding from the surface (above). 9 weeks. Dark field. 53. E: Tiny vessels from attached structure fade away on the surface of cell island. 9 weeks. Dark field. 53.



Fig. 14 Va ularized structures ttached t the inside of the basal plate A. Expanded esse attach lawlike t the surface B From the same specimen, area beside that t the preceding on Th esse ar more level with the surface. Note t ny supply vesse level w th the surf e, asculanzing three small groups of projection lightly pr truding from the surface (arrows) 9 weeks. Dark field. ×60 C Vascular loop formations level with the surface 13 weeks. Dark field ×60

course (Fig. 11) When the main vessels are more intimately adjacent to the island, i.e. level with the surface, the vessels appear very poorly filled with contrast medium in this area (Fig. 12) most likely due to endothelial hyperplasia.

When several projections are attached to the surface almost invariably the projections level with the surface are more poorly filled with contrast medium than those protruding from the surface (Fig. 13 D) —The vessels show different patterns, such as a wide-meshed net of capillaries spread over a limited area of the surface (Fig. 13 A) and loop formations (Fig. 10) Fre quently isolated vessels may be followed from the attached structure, running for some distance on the surface then fading away (Fig. 13 D E)

The preservation of isolated vessels and capillaries on the surface, seems to be in accord with the previous experience the argyrophilic reticular fibres in the walls of the vessels are more resistant to disintegration than are the collagenous fibres in the stroma (*Boe* 1967)

4 *Relationship of the Vasculature to the Basal Plate*

The similarity of the attachment of the stem villi to the basal plate and the insertion of homologous structures to the cell islands was emphasized previously —The vascularized structures attached to the inside of the basal plate, show the very same characteristics as those attached to the surface of the cell islands vascular expansions, poor vascularization of the structures adjacent to the plate, the formation of vascular loops, and isolated vessels fading away superficially Further description would be repetitive Reference is made to Fig. 14 A B C and to the legends

Discussion

The two secondary structures, the translucent villus and the syncytial band, are considered derivatives of the main type of villus. The essels of the latter appear well filled with contrast medium, whereas very little or no medium penetrates into those of the two secondary structures. As judged from the transitional forms,

it is apparent that *a marked parallelism exists between the gross transformation of the villus and the disintegration of the vascular system.*

The gradual disintegration of the vasculature seems to start in the capillary system Later the main vessels become involved. The process starts in the apical part of the villus. Accordingly villi with apices translucent and avascular are a frequent finding in the young placenta. In these transitional forms characteristic changes during the vascular disintegration were observed. In the smooth translucent, markedly swollen villus, the entire vascular system has disintegrated the structure thus appearing avascular

The relationship of the *avascular* structures to the cell islands and to the basal plate is very similar Likewise, all essential characteristics of the *vasculari ed* structures at their attachment to the surface of the cell islands and to the inside of the basal plate were similar

The growth of the chorionic tree occurs in the direction of the basal plate and the cell islands In these structures disintegrated tissue seems to become absorbed.—New branches grow out from pre-existing branches, replacing the decayed structures. Thus, growth and decay are running parallel.

The young placenta is a rapidly growing organ, in which continual development and differentiation occur The placental metabolism is extraordinary because the foetal placenta is nourished from the maternal organism, *i e* from an organism biologically and genetically different from the foetus. The material necessary for the biosynthesis of the foetal structures (anabolic processes) is available in the maternal blood conveying the material to the foetal placenta.

In what way does the placenta discharge the degradation products *i e* the result of the catabolic processes? This is the underlying main problem in the present and the two preceding papers. My hypothesis may be presented as follows. The translucent villus and the syncytial band are avascular consequently nonfunctioning structures being regarded as worn-out tissues. Obviously before these structures can be transported by the maternal blood the structures must undergo degradative processes resulting in the formation of substances of low molecular

size. Such waste products will be able to pass into the maternal circulation, from where they are excreted after further degradation if necessary

These degradative processes particularly occur in the cell islands and the basal plate. The avascular structures are gradually transformed into structureless, amorphous fibrinoid (presumably insoluble protein) revealed grossly by increasing opacity of the cell islands (Bøe 1967) The maternal enzyme systems cause the breakdown of fibrinoid into low molecular weight waste products.

This hypothesis may be supported by the evidence presented in the present and the two above mentioned papers. Conclusive evidence, however may be available when the enzyme systems in question, and the different steps in the degradation process are identified by biochemistry

SUMMARY

1 The fully vascularized main type of villus with numerous projections, becomes transformed into two secondary avascular structures, the translucent villus and the syncytial band, both structures without projections.
2. The different stages in the progressive disintegration of the vascular system were observed in the transitional forms.
3 A marked parallelism exists between the transformation of the villus, and the disintegration of the vascular system.
4 The disintegration starts in the apical part of the villus and the terminal branch.
5 The system of capillaries disintegrates before the main vessels.
6 In moderate transformation of the villus, short capillary expansions appear In advanced changes the capillary system assumes a uniformly tiny filiform appearance
7 In the translucent apical part of the villus the main vessels fade away or terminate more abruptly not infrequently in the formation of a vascular loop.
8. Foetal vessels were not observed inside the area of the cell

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DIETARY HABITS DURING PREGNANCY

A Pilot Study

BY

N.-O LUNELL, B. PERSSON AND G. STERKY

Our knowledge of the nutritional requirements during normal pregnancy is limited (WHO 1965) An important part of the management of pregnant women with different stages of diabetes mellitus is appropriate dietary advice. To determine the need for nutritional instruction and supervision in normal pregnancy a better insight into the diets of pregnant women seemed necessary

Material and Methods

Fifteen women were interviewed once during each trimester (group I) Their mean weight before pregnancy was 59 1 kg (44–70 kg) Their mean weight increase during pregnancy was 11.9 kg (7 17 kg) All but one were delivered between the 38th and 42nd week of pregnancy The birth weights and lengths of all infants fell within ±1 S D of the normal values given by *Engström* and *Sterky* 1966 Five women were between 19 and 21 years three 24–26 years, four 27–28 years and three above 30 years of age

Fifty-eight women were interviewed once during pregnancy (group II) Thirty-eight were primigravidas and twenty multigravidas. The mean weight before pregnancy was 56.6 kg (45–78 kg) and the mean weight increase was 12.1 kg (5.8–21.0 kg) All but one was delivered after a normal gestational period and only in four cases did birth weight and foetal length fall outside ±2 S D of normal values.

The physical activity or possible changes of activity of the

islands and the basal plate The majority of villi and syncytial bands attached to these structures were avascular

9 In the vascularized foetal structures attached to the surface of the cell islands vascular expansions vascular loop formations isolated capillaries, and vessels fading away on the surface were observed. Very similar changes appeared in the vascularized foetal structures attached to the inside of the basal plate.

Acknowledgements

The author owes a debt of a special gratitude to Dr Elizabeth M Ramsey whose untiring interest and helpful criticism through several years have been of vital importance for the publication of this and the preceding papers.

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Table I. *Mean Intake of Calories and Nutrients and Percentage of Recommended Standards in 15 Pregnant Women (Group I) Interviewed During Each Trimester*

Duration of Pregnancy	< 16 w		16–28 w		> 28 w	
Calories	1980	94 %	2396	104 %	20[illegible]	91 %
Protein g	65	111 %	87	111 %	72	93 %
Fat g	95	121 %	116	134 %	90	105 %
Carbohydrates g	202	73 %	234	81 %	233	91 %
Calcium mg	953	119 %	1331	102 %	1292	99 %
Phosphorous mg	1256	–	1754	–	1504	
Iron mg	12.8	65 %	18.6	93 %	12.0	60 %
Vitamin A I U	1935	65 %	4489	124 %	2249	62 %
Vitamin D μg	5 1	–	5 1	51 %	[illegible]6	[illegible]6 %
Vitamin E mg	7.3	–	9.7	–	7.5	
Thiamine mg	1.3	144 %	1.6	160 %	1.3	128 %
Riboflavine mg	1.7	131 %	2.4	152 %	2.2	135 %
Niacin mg	10 7	76 %	14.7	86 %	11.5	68 %
Ascorbic acid mg	66.0	94 %	147 0	147 %	142.0	142 %

Table II *Mean Intake of Calories and Nutrients and Percentage of Recommended Standards in 58 Pregnant Women (Group II) Each Interviewed Once During Pregnancy (Number of Women in Brackets)*

Duration of Pregnancy	< 16 w (18)		16–28 w (13)		> 28 w (27)	
Calories	2035	95 %	2185	92 %	2137	91 %
Protein g	66	114 %	[illegible]0	90 %	73	93 %
Fat g	98	123 %	104	118 %	92	106 %
Carbohydrates g	209	73 %	224	75 %	245	83 %
Calcium mg	1009	106 %	1168	78 %	1[illegible]07	91 %
Phosphorous mg	1292	–	1405	–	1533	–
Iron mg	13 6	83 %	13 4	64 %	14.2	71 %
Vitamin A I.U	2629	89 %	2278	63 %	2267	63 %
Vitamin D μg	2.5	–	3 6	36 %	2.3	23 %
Vitamin E mg	9 6	–	11.5	–	8.8	
Thiamine mg	1 3	153 %	1.2	118 %	1.5	142 %
Riboflavine mg	1 7	130 %	1 8	111 %	2.2	135 %
Niacin mg	11.5	81 %	10.2	60 %	10.6	62 %
Ascorbic acid mg	91 0	129 %	103.0	103 %	116 0	116 %

Table III *Mean Intake of Calories and Nutrients i 58 Pregnant Women (Group II) Correlated t Age*
(Number of Women in Brackets)

Age	17 19 Years (11)		20–24 Years (27)		25–29 Years (15)		30–40 Years (5)	
Calories	1851	81 %	2321	100 %	1836	84 %	2160	99 %
Protein g	66	93 %	75	105 %	64	93 %	74	99 %
Fat g	87	97 %	110	129 %	79	96 %	83	103 %
Carbohydrates g	214	69 %	242	81 %	206	74 %	265	97 %
Calcium mg	1168	76 %	1223	93 %	1222	112 %	691	72 %
Phosphorus mg	1389		1507		1384		1305	
Iron mg	11 9	68 %	14 4	77 %	10.9	62 %	19.5	103 %
Vitamin A I.U	1649	48 %	2936	87 %	2233	67 %	1754	40 %
Vitamin D μg	2.1	20 %	3.1	33 %	2.5	23 %	2.2	21 %
Vitamin E mg	8.4		10 7		9 1		8 4	–
Thiamine mg	1 4	132 %	1 5	156 %	1 1	119 %	1.3	133 %
Riboflavine mg	1.8	121 %	2 1	136 %	1 9	127 %	1.6	104 %
Niacin mg	10.3	62 %	12.6	79 %	7 7	49 %	11 7	74 %
Ascorbic acid mg	96 0	114 %	120.0	133 %	68.0	77 %	157.0	160 %

Table I. *Mean Intake of Calories and Nutrients and Percentage of Recommended Standa... in 15 Pregnant Women (Group I) Interviewed During Each Trimester*

Duration of Pregnancy	< 16 w		16–28 w		> 28 w	
Calories	1980	94 %	2396	104 %	2083	91 %
Protein g	65	111 %	87	111 %	72	93 %
Fat g	95	121 %	116	134 %	90	105 %
Carbohydrates g	202	73 %	234	81 %	233	81 %
Calcium mg	953	119 %	1331	102 %	1292	99 %
Phosphorous mg	1256	–	1754	–	1504	
Iron mg	12.8	85 %	18.6	93 %	12.0	60 %
Vitamin A I.U	1936	65 %	4489	124 %	2249	62 %
Vitamin D μg	5.1	–	5.1	51 %	[illegible]6	26 %
Vitamin E mg	7.3	–	9.7	–	7.5	
Thiamine mg	1.3	144 %	1.6	160 %	1.3	126 %
Riboflavine mg	1.7	131 %	2.4	152 %	2.2	135 %
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Calories	2035	95 %	2185	92 %	2137	91 %
Protein g	66	114 %	70	90 %	73	93 %
Fat g	98	123 %	104	118 %	92	106 %
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Calcium mg	1009	106 %	1168	78 %	1207	91 %
Phosphorous mg	1292	–	1405	–	1533	
Iron mg	13.6	83 %	13.4	64 %	14.2	71 %
Vitamin A I.U	2629	69 %	2278	63 %	2287	63 %
Vitamin D μg	2.5	–	3.6	36 %	2.3	23 %
Vitamin E mg	9.6	–	11.5	–	8.8	
Thiamine mg	1.3	153 %	1.2	118 %	1.5	142 %
Riboflavine mg	1.7	130 %	1.8	111 %	2.2	135 %
Niacin mg	11.5	81 %	10.2	60 %	10.6	62 %
Ascorbic acid mg	91.0	129 %	103.0	103 %	116.0	116 %

Dietary protein g/day

180 160 140 120 100 80 60 40 20 0

1000 1500 2000 2500 3000 3500

Total calories/day

Fig 1 The relation between intake of calories and protein in individual cases

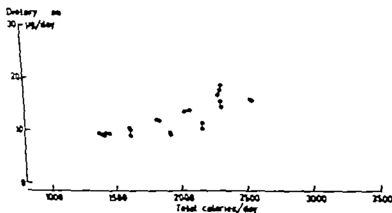


Fig 2 The relation between intake of calories and iron in individual cases

Table IV *Mean Nutritional Intake of Married and Unmarried Pregnant Women (Number in Brackets) From Group I Individual Mean Values Are Used*

Group I + II (73)		Calories	Protein g	Iron mg	Calcium mg
Unmarried	17–19 years (8)	2074	0	13.4	10 9
Unmarried	20–29 years (10)	2322	79	1 4	12[illegible]
Married	17–19 years (5)	19[illegible]8	67	10 4	1090
Married	20–40 years (50)	2102	70	13 6	11[illegible]1

Table V *Mean Nutritional Intake of Primigravidas and Multigravidas (Group I+II) From Group I Individual Mean Values Are Used*

		Calories	Protein g	Iron mg	Calcium mg
Primigravidas	(47)	2169	72	13.5	11 8
Multigravidas	(25)	2032	69	13.8	1136

pregnant women were not konwn at the interviews. The dietary intake was calculated using the 24-hour recall method (for details see *Sterky* 1962) Food consumption tables compiled by Abramson 1960 were used for calculating the nutritive values of the various food-stuffs consumed. In order to make a comparison possible all results are given as percentages of recommended standards (*Abramson* 1960) According to these recommendations a supplement of 200 calories and increases of certain nutrients are added after the second trimester

Results

In Table I the mean intake of calories and nutrients for group I is given. Corresponding values for group II are given in Table II Table III shows the mean intake in relation to age Table IV gives the nutritional intake of married and unmarried women and Table V that of primigravidas and multigravidas The correlation between the intake of calories and proteins is given in Fig. 1 and between calories and iron in Fig 2 The mean intake of protein is 33 7 g and of iron 6 7 mg per 1000 calories

Fig. 1 The relation between intake of calories and protein in individual cases

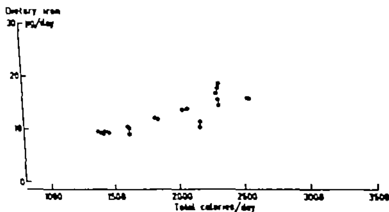


Fig. 2 The relation between intake of calories and iron in individual cases

Table IV *Mean Nutritional Intake of Married and Unmarried Pregnant Women (Number in Brackets) From Group I Individual Mean Values Are Used*

Group I + II (73)		Calories	Protein g	Iron mg	Calcium mg
Unmarried	17–19 years (8)	2074	70	13 4	10[illegible]9
Unmarried	20–29 years (10)	2322	79	15 4	12[illegible]
Married	17–19 years (5)	1968	67	10 4	1090
Married	20–40 years (50)	2102	70	13.6	1171

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In our patients there was a tendency to a moderate caloric increase between the first and second trimester best seen in group I. A smaller reduction occurred from the second to the third trimester. The increase corresponded approximately to the additional 200 calories included in the recommended standards for pregnancy.

It is noteworthy that in all groups the intake of iron was below the recommendations. The low dietary iron is of further importance in view of the fact that one third of the women did not take their prescribed drugs because of fear of side-effects or injury to the foetus. This is in accordance with the observation of *Hallberg* (1966) and also as discussed by WHO (1965).

Contrary to the assumption by WHO a deficient intake of calcium could not be demonstrated. Vitamin D and niacin were, besides iron, the only nutrients that invariably were below the recommendations. The intake of ascorbic acid was adequate and, as in other Swedish studies did not correlate with the caloric consumption.

The civil status or number of children did not seem to influence the quality of intake and the choice of food was very constant. Thus, pregnant women with low caloric consumption run the risk of getting an inadequate supply of certain nutrients.

The mean weight increase was in our series, equal to that usually found in normal pregnancy (*Lunell* 1966). During normal pregnancy there is a considerable storage of fat by the mother (*Taggart et al.* 1967). Whether this can be or ought to be influenced by dietary changes can not be answered in this study.

The results indicate that dietary instructions to pregnant women with special disorders such as diabetes mellitus must be done with great care in order to influence deep-rooted patterns in their choice of food.

SUMMARY

The dietary pattern of normal pregnant women was analysed using the 24-hour recall method. Fifteen women were followed from the first to the third trimester and fifty-eight were studied

Table VI. The Percentage Distribution of Calories among Different Foods in Groups I and II

Dried peas and beans	1.0
Vegetables	1.0
Fruits and berries	6.9
Potatoes and roots	6.0
Milk and cheese	22.2
Meat fish and eggs	21 1
Bread and cereals	20.2
Fats and oils	10 7
Sugar jam and juice	11.2

The distribution of calories among different foods is seen in Table VI. In group I there seemed to be an increase in the con sumption of milk products with increasing duration of pregnancy while a corresponding decrease in the intake of meat, fish and eggs occurred.

Group II was questioned about the intake of prescribed iron and vitamins. Eighteen out of forty women had taken iron and vitamins eight only iron, one only vitamins and thirteen women no drugs

The consumption of calories may be broken down as 14 % protein 42 % fat and 44 % carbohydrates.

Comments

The mean consumption of calories and nutrients in this series was generally satisfactory The pattern seems to be similar to that among the general population. The correlation between protein and calories and iron and calories (Figs. 1 and 2) is as constant as was earlier found for non-pregnant women (*Blix* 1965 *Carlgren* and *Cramér* 1965) The mean intake of calories (2100) in our patients was about 200 calories more than that found in 23 pregnant women by *Hallberg*, 1966 A considerably higher intake was noticed by *Stevens* and *Ohlson* 1967 in 129 pregnant women They found a satisfactory intake of almost all nutrients and a considerable improvement compared with a simi lar study conducted in the same place (Iowa City USA) in 1952.

In our patients there was a tendency to a moderate caloric increase between the first and second trimester best seen in group I. A smaller reduction occurred from the second to the third trimester. The increase corresponded approximately to the additional 200 calories included in the recommended standards for pregnancy.

It is noteworthy that in all groups the intake of iron was below the recommendations. The low dietary iron is of further importance in view of the fact that one third of the women did not take their prescribed drugs because of fear of side-effects or injury to the foetus. This is in accordance with the observation of *Hallberg* (1966) and also as discussed by WHO (1965).

Contrary to the assumption by WHO a deficient intake of calcium could not be demonstrated. Vitamin D and niacin were, besides iron, the only nutrients that invariably were below the recommendations. The intake of ascorbic acid was adequate and, as in other Swedish studies did not correlate with the caloric consumption.

The civil status or number of children did not seem to influence the quality of intake and the choice of food was very constant. Thus, pregnant women with low caloric consumption run the risk of getting an inadequate supply of certain nutrients.

The mean weight increase was, in our series equal to that usually found in normal pregnancy (*Lunell* 1966). During normal pregnancy there is a considerable storage of fat by the mother (*Taggart et al.* 1967). Whether this can be or ought to be influenced by dietary changes can not be answered in this study.

The results indicate that dietary instructions to pregnant women with special disorders such as diabetes mellitus, must be done with great care in order to influence deep-rooted patterns in their choice of food.

SUMMARY

The dietary pattern of normal pregnant women was analysed using the 24-hour recall method. Fifteen women were followed from the first to the third trimester and fifty-eight were studied

once during pregnancy In general the mean consumption of calories and nutrients was similar to that of the general population with a high intake of fat and a relatively low iron intake.

Acknowledgement

This study has been performed with financial support mainly from Semper Fond for Näringsforskning The data from the interviews were analysed with a computer at Statens Institut för Folkhalsan. Nurse Ulla Klingvall is thanked for all her help.

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THE SIGNIFICANCE OF ABSENCE OF ONE UMBILICAL ARTERY

BY

K. KRISTOFFERSEN

An abnormal number of vessels in the umbilical cord almost always means the absence of one of the umbilical arteries, aplasia arteriae umbilicalis (a.a.u.) This anomaly seems to have been mentioned for the first time by *Falloppio* (1523–1562) and the subject is described in details by *Hyrtl* (1870) who at autopsies, found some cases of a.a.u. in normal children and in malformed foetuses too.

Until the report by *Benirschke* and *Brown* (1955) a.a.u. was looked upon as an anatomical curiosity These authors analyzed 55 cases of a.a.u. and found that 27 had congenital malformations and they also noted a correlation between this anomaly and both toxaemia of pregnancy and various abnormalities of the placenta. During the last 12 years, some workers have been studying the frequency of a.a.u. the proportion of cases which have malformations, the connection with maternal diseases the pathogenesis and the clinical significance of a.a.u.

Little (1968) proposed that the finding of a.a.u. at delivery might be a useful sign leading to the possibility of immediate diagnosis of a treatable malformation of the newborn child.

The object of the present investigation is to review the literature and to analyse 41 of our own cases of a.a.u. and from this to state our present knowledge about the significance of a.a.u.

Review of the Literature

Reports f the frequency of a.a.u. and the proportion of malformations combined with a.a.u. vary considerably probably

Table I *Frequency of Aplasia Arteriae Umbilicalis and Proportions of Malformed Foetuses Reports from the Literature*

Authors	Material	Number Examined	Aplasia Art. Umbilicalis		Proportion of Mal formed Foetuses	
			Number	Per Cent	Number	Per Cent
Adler Lewenthal and Ben Adereth 1963	consecutive obstetrical	2000	19	0.95	4	22.2
Adrian 1966	consecutive obstetrical	3688	25	0 68	5	20
Angiolillo and Picinelli 1966	consecutive obstetrical	1000	2	0.20	2	100
Benirschke and Bourne 1960	consecutive obstetrical	1[illegible]00	15	1.00	7	46.6
Cairns and M Kee 1964	consecutive obstetrical	2000	20	1 00	2	10
Carrier Matteau and Jean 1966	consecutive obstetrical	4138	33	0 79	5	15.2
Froelich and Fujikura 1966	consecutive single births	26539	203	0 76	58	28 6
	of these: white	11371	139	1.22	32	23
	of these: negroes	13058	57	0 44	24	42.1
	of these: others	2110	7	0.33	2	28 6
Fujikura 1964	consecutive obstetrical	5972	[illegible]8	0.61	7	18 4
	of these, mic oscopy of ord [illegible]	1744 [illegible]	15	0 80 [illegible]		

Gornon and Koller 1964	consecutive obstetrical	1000	[illegible]	[illegible]		
Kravitz 1957	consecutive obstetrical	265	3	1.13	0	0
Lenoski and Medovy 1962	consecutive obstetrical	2500	5	0.20	1	20
Little 1961	consecutive obstetrical	2800	21	0.75	10	48
Lyon 1960	consecutive obstetrical	717	8	1.10	2	23
Papadatos and Paschos 1965	consecutive obstetrical	7886	32	0.41	10	31.2
Peckham and Yerushalmy 1965	consecutive obstetrical	5848	51	0.87	12	23.5
Thomas 1961	consecutive obstetrical	6970	27	0.40	5	18.5
Benirschke and Brown 1955	retrospective cases for 20 years		55		27	49.1
Bourne and Benirschke 1960	mostly pathological retrospect.		113		73	65
Faierman 1960	autopsies	411	11	2.7	9	81.8
Lewis 1962	autopsies	139	5	3.6	4	80
Molz 1965	autopsies	225	28	12	26	93
Seki and Strauss 1964	autopsies	1554	45	2.9	33	73.3

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Angiulillo and Picunelli 1966	consecutive obstetrical	1000	2	0.20	2	100
Benirschke and Bourne 1960	consecutive obstetrical	1500	15	1.00	7	46.6
Cairns and McKee 1964	consecutive obstetrical	2000	20	1.00	2	10
Carrier Matteau and Jean 1966	consecutive obstetrical	41.8	33	0.79	5	15.2
Froelich and Fujikura 1966	consecutive single births	26539	203	0.76	58	28.6
	of these: white	11371	139	1.22	32	23
	of these: negroes	13058	57	0.44	24	42.1
	of these: others	2110	7	0.33	2	28.6
Fujikura 1964	consecutive obstetrical	5972	8	0.61	7	18.4
	of these mi[illegible]oscopy f cord	1744	15	0.80		

(1965) and *Peckham* and *Yerushalmy* (1965) did not find any cords with a.a.u. in 108 and 66 pairs of twins, respectively In abortions *Javert* (1957) and *Thomas* (1961) find 2.4 and 2.7 per cent with a.a.u.

Among 41 pairs of twins referred to in the literature a.a.u. was found in one twin out of 39 pairs and in both of 2 pairs of dizygotic twins. Of these 41 pairs of twins 4 were monoamniotic, 14 monochorionic 19 dichorionic, of which 7 were of the same sex and in 4 cases the placentae were not described. In other words of 41 pairs of twins a minimum of 18 were monozygotic, all with discordant occurrence of a.a.u. and a minimum of 12 pairs were dizygotic twins with concordant occurrence of a.a.u. in only 2 cases. In 11 pairs of twins the zygosity could not be determined by means of the available information.

In 19 pairs of twins with discordant a.a.u. the birthweights were known. The smaller twin was the one with a.a.u. in 16 cases and the bigger twin in 1 case (*Benirschke* and *Brown* 1955 *Thomas* 1961 *Seki* and *Strauss* 1964) while in 2 cases of monoamniotic twins the birthweights were equal (*Moestrup* 1969 *Wharton et al.* 1968) These 4 monoamniotic twins had no malformations.

German et al (1962) *Lenoski* and *Medovy* (1962) *Lewis* (1962) *Uchida Bowman* and *Wang* (1962) *Henrichs* and *Allen* (1963) and *Seki* and *Strauss* (1964) have together found 25 patients with autosomal trisomy of whom 14 also had a.a.u. *Kajii et al* (1963) found a.a.u. in two out of four thalidomide malformed babies, and *Dunn Fisher* and *Kohler* (1962) reported a.a.u. in one of two dizygotic twins both with severe malformations caused by thalidomide. *Molz* (1965) mentioned that most of the children with a.a.u. and skeletal malformations in his autopsy material date from the period when thalidomide was in use in Western Germany but there are not any prospective studies to prove a causal relationship between a.a.u. and the use of thalidomide

The ratio between males and females with a.a.u. is 1/1 (*Benirschke* and *Brown* 1955 *Froelich* and *Fujikura* 1966) Three authors (*Hyrtl* 1870 *Seki* and *Strauss* 1964 and *Molz* 1965) find a preponderance of boys among the children with a.a.u.

because different methods have been used for collecting cases and for examining the cords But even if a consecutive collection of cords from one obstetrical department is studied, it is impossible to rule out some selection because some departments admit all deliveries while others have a relatively high proportion of complicated cases

Table I shows the reported findings over the last 12 years with a description of the method of collecting cords the number and incidence of a.a.u. and the proportion of malformations. The incidence of a a u. in reports from obstetrical departments varies from 0 2 to 1 22 per cent, and the corresponding results from pathological departments, based on autopsies from 2.7 to 12 per cent. In very rare cases it is stated that the two umbilical arteries join one another near the placenta, (*Hyrtl* 1870 *Little* 1961 *Seki* and *Strauss* 1964) and fusion of the two umbilical arteries to form a single trunk within the foetal abdomen, has also been seen at autopsy (*Seki* and *Strauss* 1964) However the most common abnormal finding is that one of the umbilical arteries left or right with equal frequency is absent from its origin. (*Bourne* and *Benirschke* 1960 *Faierman* 1960 *Bridges* and *Morton* 1964 *Molz* 1965) and the iliac and femoral arteries on the same side may be hypoplastic (*Kajii Shimohara Ki chuchi Dohmen* and *Akichika* 1963)

The proportion of malformations associated with a.a.u. varies from 0 to 100 per cent, but in the largest series the figure ranges from 15 to 48 per cent, and the proportion is even greater (from 49 1 to 93 per cent) in autopsy material.

The malformations may occur in all parts of the body However some authors find a preponderance in some systems for example the gastrointestinal and skeletal systems (*Fujikura* 1964 *Mol* 1965) the cardiovascular system (*Seki* and *Strauss* 1964) the central nervous system and the heart (*Benirschke* and *Brown* 1955) and the skeletal system alone (*Froelich* and *Fujikura* 1966) Almost all authors find that in most cases there are multiple malformations in different organs in the same child.

Some authors find that the incidence of a.a u. in twins is greater than normal namely 3 5 to 4 per cent (*Benirschke* and *Bourne* 1960 *Thomas* 1961) while *Papadatos* and *Paschos*

(1965) and *Peckham* and *Yerushalmy* (1965) did not find any cords with a.a.u. in 108 and 66 pairs of twins, respectively In abortions *Javert* (1957) and *Thomas* (1961) find 2.4 and 2.7 per cent with a.a.u.

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but this was in selected autopsy material in which there are usually more males.

Some authors have noticed that children with a.a.u. have lower birthweights than normal (*Benirschke* and *Brown* 1955 *Adler et al* 1963 *Carrier et al* 1966) However *Seki* and *Strauss* (1964) find their birthweights as expected for the gestational age when allowance is made for the smaller weights of children with multiple malformations.

Froelich and *Fujikura* (1966) found the incidence of a.a.u. greater in white people (1.22 per cent) than in negroes (0 44 per cent) in a prospective study collected from 12 clinics, some treating mostly white patients and others mostly negroes, and including a total of 11,371 and 13 058 mothers respectively Virtually the same figures were reported by *Peckham* and *Yerushalmy* (1965) 1 11 per cent a.a.u. on examination of 3597 white babies and 0 43 per cent of 1 167 negroes.

The perinatal mortality is high in cases of a.a.u. (13–30 per cent) primarily because of the many lethal malformations, but there is also an increased mortality among children without detectable anomalies. (*Little* 1961 *Fujikura* 1964 *Froelich* and *Fujikura* 1966 *Peckham* and *Yerushalmy* 1965)

As to the age of the mothers of the children with a a.u. *Benirschke* and *Brown* (1955) found that 21 of 44 (48 per cent) were more than 30 years and *Froelich* and *Fujikura* (1966) find a slight preponderance of mothers over 20 years of age Other authors however could not find any difference from the normal age distribution (*Little* 1961 *Cairns* and *McKee* 1964 *Seki* and *Strauss* 1964) *Little* (1961) found a slight preponderance of multiparae but others report normal distribution with regard to parity

A significant preponderance of diabetes mellitus is found among mothers of children with a.a u. *Seki* and *Strauss* (1964) had an incidence of 14 per cent (7 50) and *Froelich* and *Fujikura* (1966) 6 4 per cent (13/203) A slightly greater occurrence than expected but not significant, is found in mothers suffering from neuro-psychiatric diseases (*Fujikura* 1964 *Froelich* and *Fujikura* 1966)

Benirschke and *Brown* (1955) found that 30 per cent of the

mothers had preeclampsia and polyhydramnios occurred 4 times more than expected in their series. They also found that 18 of 46 placentae (39 per cent) appeared to have anomalies. These were mostly circumvallate placentae, velamentous insertions of the cord and infarcts. *Seki* and *Strauss* (1964) found that 6 of their 52 cases had a velamentous insertion of the cord and 7 of 52 placentae weighed less than expected in relation to the weight of the child, and the same correlation was found in the large series published by *Froelich* and *Fujikura*.

Seki and *Strauss* (1964) found that 15 out of 50 mothers of children with a.a.u. had a history of one or more abortions and *Adrian* (1966) reported that, out of 25 such mothers, 17 had a history of 33 pregnancies only 16 of which resulted in a mature living child.

In the consecutive obstetrical series four different methods of demonstrating the vessels in the cord have been used

1) Inspection of the cut-end of the fresh umbilical cord at birth. This method has been most frequently used, and has yielded an incidence of a.a.u. ranging from 0.2 to 0.8 per cent.

2) Naked eye inspection of a slice of the umbilical cord after fixation by formalin or "fixative solution (*Cairns* and *McKee* 1964 *Peckham* and *Yerushalmy* 1965)

3) Microscopic examination of all cords (*Benirschke* and *Bourne* 1960 *Lyon* 1969 *Froelich* and *Fujikura* 1966) By these two methods the incidence of a.a.u. is found to be somewhat higher (0 76–1 1 per cent)

4) Inspection of the umbilical cord after fixation in glacial acetic acid (*Kravitz* 1967) This author only examined 265 cords by this method and found 3 with a.a.u. (1 13 per cent) but he claims that with this treatment the cord becomes translucent and rubbery in consistency so that the vessels are more easily seen.

Own Material and Methods

A study has been made of 41 children with a.a.u. Of these, 16 hav previously been referred in a lecture in Dansk Selskab for Obstetrik og Gynækologi on the 24th of January 1964 These

are collected in a two year period from The Maternity Department B Rigshospitalet, Copenhagen (17 cases) The Department of Obstetrics and Gynaecology D Odense County and City Hospital, Odense (16 cases) and The Municipal Maternity Clinic in Odense (8 cases)

Two different methods have been used for the examination of the cords

1) From 9/1 1964 to 23/12 1964 1129 cords were fixed in formalin and examined later by the author In this way 13 cases (1 15 per cent) of a.a.u. were demonstrated.

2) The remaining 28 cases of a.a.u. were found by a routine inspection of fresh cords carried out by the staff of the various departments 7622 children were born during the period of the examination. This gives an incidence of 0.37 per cent with a.a.u. The difference between the results obtained by the two methods is significant ($p < 0\ 01$) Twenty-eight of the 41 cords with a.a.u. were examined microscopically while the remaining 13 were only inspected macroscopically

In the whole series there were 106 pairs of twins and the cords from 26 of these were examined after fixation in formalin.

Results

Table II a b and c show how the mothers of the 41 children are distributed in relation to age gravidity and parity 11 of the 41 (26 8 per cent) were more than 30 years of age and 7 (17 1 per cent) had a history of 4 or more pregnancies. This distribution does not differ from that found in a study of all mothers delivered in Odense County and City Hospital during the period of the examination.

Prior to the pregnancy studied the 41 mothers had a history of 58 pregnancies with results as shown in Table III. It is seen that their obstetrical histories are loaded with 13 spontaneous abortions (22.4 per cent) and a total foetal loss of 31 per cent, because 5 children died within 3 weeks of birth. The 5 legal abortions have been excluded. The 18 unsuccessful pregnancies were shared by 14 mothers while 27 mothers had not lost any children

Table II a. Distribution *of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicalis by Age*

Age) ars	≤20	>20≤25	>25≤30	>30≤35	>.5≤40	>40	total
Number	5	16	9	8	1	2	41

Table II b. Distribution *of 41 Mothers of 41 Children with Aplasia Arteriae* Umbilicalis by Gravidity

Pregnancy	I	II	III	>IV	total
Number	16	8	10	7	41

Table II Distribution *of 41 Mothers of 41 Children with Aplasia Arteriae* L mbilicalis *by Parity*

Parity	I	II	III	>IV	total
Number	21	8	7	5	41

Table III *Results of the 58 Pregnancies Prior to 41 Pregnancies Which Re-ul d in Children with Aplasia Arteriae Umbilicalis*

	Spontaneous abortions	Legal abortions	Perinatal deaths	Li ing children	Total
Number	13	5	5	35	58
Per ent	22 4	8 6	8 6	60 4	100

Eight of the mothers had 11 diseases and 14 mothers had 17 complications of their pregnancies as shown in Tables IV a and b The four cases of insulin treated diabetes mellitus are 4 times more than expected. Among anomalies of the placenta infarcts were found in 8 cases and velamentous insertion of the umbilical cord in 2 cases. Three of the mothers had a family history of congenital malformations. In one family four girls had congenital dislocation of the hip in another family one child had congenital heart disease and one had died when 8 months old from an unknown cause In a third family the mother had previously gi en birth to a hydrocephalic child (no. 6 in Table VI)

One of the 41 children, a second twin (935 g) was a stillborn

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In the whole series there were 106 pairs of twins and the cords from 26 of these were examined after fixation in formalin.

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Table II *Distribution of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicalis by Age*

Age: years	≤20	>20≤25	>25≤30	>30≤35	>35≤40	>40	total
Number	5	16	9	8	1	2	41

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Pregnancy	I	II	III	>IV	total
Number	16	8	10	7	41

Table II *Distribution of 41 Mothers of 41 Children with Aplasia Arteriae Umbilicalis by Parity*

Parity	I	II	III	>IV	total
Number	21	8	7	5	41

Table III *Results of the 58 Pregnancies Prior to 41 Pregnancies Which Resulted in Children with Aplasia Arteriae Umbilicalis*

	Spontaneous abortions	Legal abortions	Perinatal deaths	Living children	Total
Number	13	5	5	35	58
Per cent	22.4	8.6	8.6	60.4	100

Eight of the mothers had 11 diseases and 14 mothers had 17 complications of their pregnancies as shown in Tables IV a and b. The four cases of insulin treated diabetes mellitus are 4 times more than expected. Among anomalies of the placenta infarcts were found in 8 cases and velamentous insertion of the umbilical cord in 2 cases. Three of the mothers had a family history of congenital malformations. In one family four girls had congenital dislocation of the hip. In another family one child had congenital heart disease, and one had died when 8 months old from an unknown cause. In a third family the mother had previously given birth to a hydrocephalic child (no. 6 in Table VI).

One of the 41 children, a second twin (935 g) was a stillborn

Table IV *Mothers Diseases Complications in Pregnancy and Anomalies of Placenta in 41 Cases of Aplasia Arteriae Umbilicalis*

Eight Mothers with Following Diseases.	
Diabetes mellitus	4
Epilepsy	2
Mitral stenosis	1
Pulmonary TB	1
Psycho-neurosis	1
Uterine fibroids	1
Jaundice of pregnancy	1

a

Fourteen Mothers with Following Complications in Pregnancies
Preeclampsia
Essential Hypert.
Proteinuria
Oedema
Threatened abortion
Bleeding of unknown cause
Anaemia
Rhesus iso-immunization
ABO-iso-immunization
Hydramnios

b

Anomalies of Placenta.	
Infarcts	8
Velamentous insertion of the cord	2
Placenta marginata	1
Placenta praevia	2
Vasa praevia	1
8-shaped placenta	1
Retained placenta	1

c

monster whose sex could not be determined otherwise there were 20 males and 20 females

The birthweights of the children are distributed as shown in Table V Two were immature (≤1000 g) 14 premature and 24 weighed more than 2500 g Of the 29 children born of mothers without diabetes mellitus and in whom the gestational age was known, 9 had a birthweight higher and 20 lower than that calculated from the formula of Streeter The average weight of the 29 children was 288 g less than expected The fate of the 41 children is also analysed in Table V 5 were stillborn, 6 died within 3 weeks so the mortality was 26 8 per cent. Eleven (26 8 per cent) had congenital malformations The malformations are described briefly in Table VI together with the mother s age parity associated diseases and complications It is remarkable

Table V *Distribution in Weight-Classes and Its Correlation to the Fate of 41 Children with Aplasia Arteriae Umbilicalis*

Weight in Grams	≤1000	>1000≤2500	>2500	Unknown	Total
Total number	2	14	24	1	41
Living	0	6	24	0	30
Dead	0	5	0	1	6
Stillborn	2	3	0	0	5
Malformations	1	8	1	1	11

that only one of the 24 mature children had malformations (no. 4) while 8 of 14 premature and 1 of 2 immature babies were malformed. Only 2 of the 11 malformed babies are alive (no. 4 and 11) Seven had multiple anomalies involving various systems, and 5 of these were severe enough to be incompatible with life. No malformations were found in the 2 stillborn babies but only one of them had autopsy

There was only one second twin with a.a.u. among 106 pairs of twins (no. 3 in Table VI)

In two cases (no. 1 and 2) the malformation was diagnosed shortly after birth because of the finding of a.a.u. which led to a thorough examination of the babies, including the passage of an oesophageal catheter a chest X ray and an E.C.G.

The 2261 cords from Odense County and City Hospital were examined routinely both at the placenta and at the umbilicus and in no case was there any discrepancy between the two observations

The incidence of a.a.u. in relation to the seasons of the year is mentioned by *Peckham* and *Yerushalmy* (1965) who found that the last menstrual period of the mothers giving birth to children with a.a.u. was in the quarter July-September in 24 out of 51 mothers which was more than twice as often as in each of the other three quarters of the year In the present investigation the time of conception could be determined in 39 mothers and the distribution in the four quarters of the year was 8-12-12 and 7 respectively so that 24 of the 39 fell in the half year April-September

Table IV *Mothers Diseases Complications in Pregnancy and Anomalies of Placenta in 41 Cases of Aplasia Arteriae Umbilicalis*

Eight Mothers with Following Diseases:	
Diabetes mellitus	4
Epilepsy	2
Mitral stenosis	1
Pulmonary TB	1
Psycho-neurosis	1
Uterine fibroids	1
Jaundice of pregnancy	1

a

Fourteen Mothers with Following Complications in Pregnancies:
Preeclampsia
Essential Hypert.
Proteinuria
Oedema
Threatened abortion
Bleeding of unknown cause
Anaemia
Rhesus iso-immunization
ABO-iso-immunization
Hydramnios

b

Anomalies of Placenta.	
Infarcts	8
Velamentous insertion of the cord	2
Placenta marginata	1
Placenta praevia	2
Vasa praevia	1
8-shaped placenta	1
Retained placenta	1

c

monster whose sex could not be determined, otherwise there were 20 males and 20 females

The birthweights of the children are distributed as shown in Table V Two were immature (≤ 1000 g) 14 premature and 24 weighed more than 2500 g Of the 29 children born of mothers without diabetes mellitus and in whom the gestational age was known, 9 had a birthweight higher and 20 lower than that calculated from the formula of Streeter The average weight of the 29 children was 288 g less than expected The fate of the 41 children is also analysed in Table V 5 were stillborn, 6 died within 3 weeks so the mortality was 26 8 per cent. Eleven (26 8 per cent) had congenital malformations The malformations are described briefly in Table VI together with the mother's age parity associated diseases and complications It is remarkable

Umbilicalis: Gestational Age Weight Length Sex and Their Mothers Age Gravidity Complications in Pregnancy

Weight Grams	Length cm	Sex	Mothers Age	Gravidity	Parity	Diseases and Complications in Pregnancy
1550	44	F	23	I	I	Haemorrhage in pregnancy preeclampsia, velamentous insertion of the cord, placental infarct
2400	49	M	22	II	II	Placental infarct
935	22	?	19	III	II	Eight-shaped placenta
3350	53	F	25	II	II	Diabetes mellitus and epilepsy
1180	34	M	27	III	II	Diabetes mellitus preeclampsia, hydramnios
1330		F	26	III	III	Well, but has previously given birth to child with hydrocephalus and spina bifida
2400	45	M	20	I	I	Psycho-neurosis treated by different drugs during pregnancy
2000	44	F	25	II	I	Threatened abortion in pregnancy
2250	49	F	25	I	I	0
		M	28	IV	IV	Retained placenta
1900	46	M	35	V	III	0

Table VI. *Records of Observed Malformations in 11 Children with Aplasia Arteriae* ... *Parity Diseases and*

No	J	No	Description of Malformations and Fate of Child	Gestational Age in Days
1	B	377/62	Oesophageal atresia operated died 11 days old	287
2	B	1784/62	Endomyocardial fibroelastosis coarctation of the aorta aplasia arteriae umbilicalis intraabdominalis died 3 days old	252
3	B	2078/62	Stillborn monster second twin, 3 limbs and small excrescences instead of head cystic kidney	180
4	B	2279/62	Single harelip alive 4 years old, well	259
5	B	1729/63	Stillborn anencephalic cervical rachischisis splenomegaly hypoplasia of the suprarenal glands	246
6	B	2084/63	Stillborn anencephalic club-feet hypoplasia of the suprarenal glands (1 g)	189
7	A	854/63–64	Housden's syndrome: atresia mm. abdominis malrotation of gut hypoplastic kidneys distended bladder vesico-umbilical fistula, club-feet died 20 days old	263
8	A	220/6_–64	Tricuspid stenosis hypertelorism syndactyly died 2 days old	245
9	A	480/63–64	Hydrocephalus anophthalmia died soon after birth	270
10	D	222/64–65	Stillborn with omphalocele club-feet harelip	266
11	D	1924/64–65	Branchial fistula alive Two years old he was examined in paediatric department Diagnosis. branchial fistula nasal catarrh nummular eczema enlarged tonsils and adenoids and perhaps mental retarded	235

...ducals: Gestational Age Weight Length Sex and Their Mothers Age Gravidity ...plications in Pregnancy

...ight ...ms	Length cm	Sex	Mothers: Age	Gravidity	Parity	Diseases and Complications in Pregnancy
50	44	F	23	I	I	Haemorrhage in pregnancy preeclampsia velamentous insertion of the cord, placental infarct
400	49	M	22	II	II	Placental infarct
925	22	?	19	III	II	Eight-shaped placenta
3050	53	F	25	II	II	Diabetes mellitus and epilepsy
1180	34	M	27	III	II	Diabetes mellitus preeclampsia, hydramnios
1330	?	F	28	III	III	Well, but has previously given birth to child with hydrocephalus and spina bifida
2400	45	M	20	I	I	Psycho-neurosis treated by different drugs during pregnancy
2000	44	F	25	II	I	Threatened abortion in pregnancy
2250	49	F	25	I	I	0
		M	38	IV	IV	Retained placenta
1900	46	M	35	V	III	0

Follow up studies

Questionnaires were answered by the mothers of 24 of the 30 children, who were alive on leaving hospital They were asked about the children s general health and whether they had suf fered from any diseases up to the age of 2–5 years. 15 children had been quite healthy since leaving hospital and were thriving

Of the other 9 children, 4 had had many upper respiratory tract infections one child was very thin and would not eat, one child had a history of otitis and fever convulsions was slender and would not eat one was treated in the radiological depart ment for a haemangioma (1 8×1.2 cm) on the abdominal skin just below the right costal margin, and one had been taken into the hospital for an inguinal hernia but the hernia disappeared spontaneously The last child was taken into the paediatric department for examination as a result of the answer on the questionnaire and the diagnoses are listed in Table VI (no 11)

From these questionnaires it is not possible to reach a firm conclusion whether these children s health and growth are any worse than normal. As quite a lot of them (6) are said to have recurrent infections there are good reasons for paediatric follow up throughout childhood.

Discussion and Conclusions

The incidence of a.a.u has been found to be significantly higher on macroscopic or microscopic examination after fixation than by routine inspection of fresh cords at birth. This is confirmed in the present investigation where 1 15 per cent and 0.37 per cent a.a u. respectively were found by these two methods. It is concluded that the best way to pick up all cases of a.a.u. is to examine all cords macroscopically or microscopically after fixation in formalin or glacial acetic acid. In the present series fixation in formalin has made the vessels stand out clearly However comparative studies in the same umbilical cord (Fig 1) suggest that the glacial acetic acid method is even better giving a translucent cord with the course of the vessels very clearly seen.

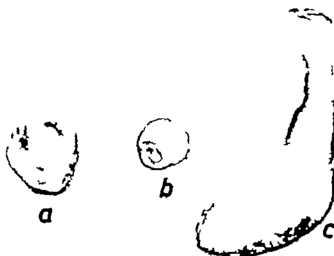


Fig. 1 Umbilical cord treated with ( ) formalin-alcohol (b) and ( ) treated with glacial acetic acid.

By these methods the incidence of a.a.u. has been found to range from 0 76 to 1 15 per cent. Although the patients in most obstetrical departments are somewhat selected to include more abnormal pregnancies than in the general population, it seems reasonable to estimate that the incidence of a.a.u. is between 0 5 and 1.0 per cent. The proportion of associated malformations is 25 to 50 per cent. The incidence of congenital malformations in the general population is about one per cent, therefore between 1/4 and 1/2 of children with severe malformations lack one umbilical artery

Abnormalities of the placenta especially velamentous insertion of the cord and infarcts are found in association with a.a.u. more frequently than is expected. Maternal diabetes mellitus is found 4 times more often than expected in the present series, and this agrees with previously published results. There are more mothers than normal with illnesses although no single

disease is significantly increased, apart from diabetes mellitus. The incidence of preeclampsia and haemorrhage in pregnancy is a little more than normal

The present series includes 106 pairs of twins and only one child with a a.u was found. This agrees with the findings of *Papadatos* and *Paschos* (1965) and those of *Peckham* and *Yerushalmy* (1965) while the increased incidence of a.a.u. among twins referred to by *Benirschke* and *Bourne* (1960) and *Thomas* (1961) could not be confirmed. It is not possible to explain this discrepancy

As to the cause of a.a u. and the many associated malformations *Benirschke* and *Brown* (1955) have proposed the following theories a) a a u. might be one of a number of malformations in the same child and with the same cause b) it is also possible that the absence of one artery might cause an increased resistance to the umbilical blood flow with possible foetal hypoxia and consequently multiple malformations They emphasise that the causative factor must be present for long periods of embryonic development in order to explain so many varied malformations and the fact that there is no familial occurrence of a.a.u. excludes a genetic cause

The discordance in monozygotic twins and the seasonal variations in the occurrence of a.a.u. indicate that environmental factors are more important. On the other hand the high incidence of miscarriages in the obstetrical history of the mothers bearing children with a.a.u. might indicate a hereditary disposition to congenital malformations

Whether a a.u is the cause of the associated malformations or a part of these with a separate common cause the existence of a a.u must indicate some exogenous factor By exogenous is meant a factor outside the foetus and its placenta and might for instance be abnormal metabolism in the mother or drugs taken by her In the present series congenital malformations were found in 9 out of 16 children with a a u and a birthweight ≤2500 g, but only one out of 24 with a birthweight >2500 g had a minor malformation (harelip) These findings agree very well with those of *Seki* and *Strauss* who state that the birth weights of children with a.a.u were as expected for the gesta

tional age except for those with multiple malformations. So it may be concluded that children with both a.a.u. and low birth weight have a greater incidence of malformations than children with a.a.u. and normal birthweight. Among the twins recorded in the literature it was found that the twin with a.a.u. had a lower birthweight than the other one. These facts might indicate that a.a.u. could cause impaired nourishment of the foetus with resulting low birthweight and when this impairment is marked, it might also be the cause of multiple malformations.

Rahbek Sorensen (1953) found in his investigation of hypospadias in twins, that in 5 cases where this anomaly was discordantly present, the twin with hypospadias had a lower birthweight than the other twin. No examination of the cord vessels was done in his series, but it is interesting to find this similarity to the findings in a.a.u. because it supports the theory that an impaired supply of oxygen and/or nutrition to the foetus may be accompanied by or perhaps be the cause of malformations.

The clinical significance of the finding of a.a.u. as a means of diagnosing a congenital malformation is much reduced by the fact that the malformations are most often severe and multiple. However in a few cases, (twice in the present series) the finding turns out to be of practical clinical importance.

In experimental teratology in animals and in prospective studies of human malformations the demonstration of a.a.u. is supposed to be of significance as an indicator of exogenous causative factors. However the problem is very complicated because we must allow as emphasized by *Bro-Rasmussen* and *Hansen* (1961) for the possibility that exogenous factors may work on a background of individual gene-patterns which govern a greater or lesser disposition to malformations.

Follow-up of children with a.a.u. has been done by a few authors. *Benirschke* and *Bourne* (1960) on examination of 10 children at the age of 6 months found no special disorders, and *Froelich* and *Fujikura* (1966) examined 99 children at the age of 12 months The latter authors point out that there were no uro-genital disorders except for three cases of hypospadias. *Fujikura* (1964) found that three out of 16 affected children were retarded in their movement at 12 months. *Little* (1961) mentions

that 8 out of 15 living children had physical findings compatible with congenital abnormalities two of these showing congenital heart disease. The results in the present follow up study which showed that 6 out of 24 children were susceptible to infections, suggest that there is good reason in the future to follow up these children in paediatric clinics and observe their motor and mental development. It might be of particular interest to examine the legs to see if they are equally developed remembering that unilateral hypoplasia of iliac and femoral arteries may be found in these children.

SUMMARY

A review of the literature of the last 12 years on aplasia arteriae umbilicalis (a.a.u.) is presented. Together with 41 personal cases the reports are analysed and the following conclusions made

1 Aplasia arteria umbilicalis occurs in 0.5 to 1 per cent of all cords but it is 2 to 3 times more frequent in aborted and premature foetuses than in mature babies. The incidence in girls and boys is the same

2. Congenital malformations are found in 25 to 50 per cent of children with a.a.u. They tend to be multiple and lethal and are faremore common in premature babies

3 The incidence of a.a.u in twins is found by some authors to be increased to about 4 per cent, but in other series including the present one no such increase is found. A.a.u. is perhaps a little more frequent in monozygotic than dizygotic twins but was not found to be concordant in any of 18 pairs of monozygotic twins The twin with a.a.u. practically always has lower birthweight than the other one

4 In children of diabetic mothers the incidence of a.a.u. is increased 4 to 6 times Medical diseases as a whole but perhaps especially neuro-psychiatric and cardiac disorders are found more commonly among mothers bearing children with a.a.u.

5 Complications of pregnancy such as preeclampsia, hypertension and ante-partum haemorrhage often due to placenta praevia, as well as other abnormalities of the placenta seem to be more frequent than normal.

6 Demonstration of the umbilical vessels is most reliable if a piece of all cords is fixed in formalin, or better in glacial acetic acid, for future examination, as the vessels are then clearly seen by the naked eye.

7 Follow-up of the development and health of the children born with a.u.u., and without other visible anomalies, has not given consistent results and therefore this problem needs further investigation.

8 The high incidence of congenital malformations in association with a.u.u. is supposed to indicate that these malformations are caused by exogenous factors in the environment of these children.

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THE INFLUENCE OF PROPHYLACTIC EXTERNAL CEPHALIC VERSION ON THE INCIDENCE OF BREECH DELIVERY

A Retrospective Study

BY

JOHANNES E. BOCK

It is well known that the delivery of a breech presentation involves a greater risk to the foetus than that of a cephalic presentation. A part of this increased risk is due to the fact that more premature babies are delivered as breech presentations, but even when these are excluded the risk is still greater. The perinatal mortality rate for infants weighing over 2,500 g is higher in breech presentations (*Cannel* and *Dodek* 1934, *Fischer Rasmussen* and *Trolle* 1967) and the incidence of mental and motor retardation is greater among children delivered in this way (*Bishop, Israel* and *Briscoe* 1965).

In view of this increased risk to the foetus it would seem reasonable to carry out prophylactic external cephalic version in order to convert a breech presentation to the less dangerous vertex presentation before labour commences.

Many authors favour this procedure and consider that it results in a decrease in the incidence of breech deliveries (*Jokela* 1949, *Dalley* 1952, *Kuhnel* 1954, *v. Friesen* 1957, *Betscher* and *Townsend* 1960). *Von Friesen* (1957) finds a 3.03 per cent incidence of breech delivery (among 4 448 single births with a birth weight of more than 2,500 g) when external version is not performed and a 1.70 per cent incidence (among 4 181 single births) when external version is performed. This decrease of 1.33 per cent is statistically significant.

Other authors have questioned the value of the prophylactic external cephalic version (*Olow* and *Posse* 1950, *Brosset* 1956, *Morgan* 1962, *Greenhill* 1965). *Brosset* (1956) states that ex

ternal version can only be performed in cases where spontaneous version would have taken place and, therefore he finds external version of breech presentations of little value. *Morgan* (1962) is of the opinion that the foetal mortality rate cannot be reduced by substituting the head for the breech because the version will carry with it a certain foetal mortality *Greenhill* (1965) finds only a slight difference in the mortality of vertex and breech presentations and, therefore he seldom performs external version.

The incidence of breech presentation at birth is usually given as 3–4 per cent. In the 32nd week 25 per cent of breech presentations have been found (*Vartan* 1945) Spontaneous version is responsible for the decrease According to *Vartan* (1945) *Jokela* (1949) and *Kühnel* (1954) spontaneous version after the 34th week occurs so rarely that it may be assumed that the optimum time for external version is from the 34th week on wards

External version without anaesthesia is preferable. In *Fell's* (1953) series foetal loss due to version was 2 per cent when the patients were anaesthetized, whereas there were no foetal deaths when the external versions were carried out without anaesthesia. When the patient is conscious only gentle manipulations will be tolerated and this is a safeguard against accidents which mostly result from the use of excessive force The risks to the foetus are partial separation of the placenta the onset of premature labour following rupture of the membranes and prolapse of the cord. There is virtually no risk to the mother Cases of rupture of the uterus as a consequence of version have been reported but this happens very seldom and the presence of a uterine scar due to previous Caesarean section or myomectomy does not contraindicate the performance of external version (*Fell* 1953)

External version is contraindicated in patients with a history of antepartum haemorrhage in patients with hypertension (*Fell* 1953) and of course in patients where elective Caesarean section is planned and in multiple pregnancies According to *Greenhill* (1965) uterine scars are a contraindication too

The incidence of successful prophylactic external version varies *Brosset* (1956) found that only 30 per cent of the ver

sions performed were successful. This incidence is low compared with that reported by other authors (*Jokela* 1949 *Fell* 1953 *Kühnel* 1954 *Friedlander* 1966) The majority of these authors were successful in about 80 per cent or more of their cases. Successful version becomes less likely as term is approached, presumably for the same reasons that spontaneous version becomes less frequent. The increasing size of the foetus and the tendency to fixation of the presenting part are probably the main factors concerned. Apart from the gestation period the factors which make successful version less likely include extended legs, scanty liquor amnii a thick abdominal wall, and an irritable uterus.

Material

In order to get an impression of the influence of prophylactic external cephalic version on the incidence of breech presentation two 5-year periods have been analysed and compared.

During the first period January 1 1957–December 31 1961 there was not any special department of obstetrics in the Esbjerg area. No attempts at external version were made. Most deliveries took place in the patients homes. 4,316 consecutive single births weighing more than 2,500 g were collected during this period.

During the second period April 1 1962–March 31 1967 prophylactic external version was performed at the Department of Obstetrics in the same area. 6 058 single births weighing more than 2,500 g took place at the Department during this period. Few deliveries in the area still took place in the patients homes. Among these there were not any breech presentations weighing more that 2,500 g. The incidence of breech delivery in the area may therefore be somewhat lower than the incidence in the Department.

Attempts at version were performed without anaesthesia from the 34th week onward up to one day before term.

Results

During the period 1957–1961 4 316 single births weighing more than 2,500 g were registered. The incidence of breech delivery was 3 15 per cent (Table I)

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The incidence of successful prophylactic external version varies *Brosset* (1956) found that only 30 per cent of the ver

During the period 1962–1967 the incidence of breech delivery was 2.05 per cent (Table II)

The difference between the incidence of breech delivery when version was not performed and that when version was performed is 1 10 per cent. The decrease is statistically significant ($p < 0.001$) The perinatal mortality rate for cephalic presentations over 2,500 g in the last 5-year period was 1 13 per cent. The perinatal mortality rate for the 124 deliveries of breech presentations was 4.91 per cent (Table III)

One of the six foetal deaths was due to a lethal malformation (anencephaly) The causes of the other foetal deaths were prolapse of the cord (2) intracranial haemorrhage (1) and unknown (2) The corrected mortality rate is 4.04 per cent (Table III) For cephalic presentations the corrected mortality rate is 1 05 per cent.

340 attempts at external version were performed in 296 patients Two three or four attempts were made if version was not successful or if the presentation reverted after a successful version. The results are illustrated in Table IV

In the remaining 68 cases of breech deliveries attempts at version were not performed. 31 patients were admitted to the Department in labour without any antenatal care at the Department. 32 patients had a cephalic presentation at the antenatal clinic six to three weeks before term, but there may be a possibility of incorrect diagnosis since no radiographs were taken. Spontaneous version to a breech presentation is also a possibility In 5 cases external version was contraindicated. 2 patients had antepartum haemorrhage and in 3 patients elective Caesarean section was planned because of the breech presentation in association with previous classical Caesarean sections (2) and pelvic contraction (1) Previous lower segment Caesarean section was not considered as a contraindication to the performance of external version.

In 3 cases external version was followed by complications and one of these resulted in foetal death

IV gravida 28 years old 4 weeks before the expected date of delivery the patient was found to have breech presentation. External version was performed 3 days after the version foetal movements disappeared, and labour

Table I. *Single Births over 2,500 g 1957–1951*

Total deliveries	4,316
Breech presentations	136
Incidence of breech delivery (per cent)	3 15

Table II. *Single Births over 2,500 g. 1952 1957*

Year	1952/63	1963/64	1964/65	1965/66	1966 67	Total
Total deliveries	848	1 105	1 199	1,373	1,533	6,053
Breech presentations	21	26	23	31	23	1 4
Incidence of breech delivery (per cent)	2.48	2.44	1.92	2.26	1.50	2.05

Table III. *Perinatal Mortality Rate 1962–1957*

	Breech Presentation	Cephalic Presentation
Total number of deliveries weighing over 2,500 g	124	5,928
Perinatal mortality (per cent)	4.91 (6/124)	1 13 (67 5 928)
Corrected mortality (per cent)	4 04 (124)	1 05 (62 5 928)

Tabl IV *Attempts at Version*

	Number of patients	Per Cent	Number of Attempts at Version
Successful	240	81 1	262
Unsuccessful	56	18 9	8
Total	296	100 0	340

During the period 1962–1967 the incidence of breech delivery was 2.05 per cent (Table II)

The difference between the incidence of breech delivery when version was not performed and that when version was performed is 1.10 per cent. The decrease is statistically significant ($p < 0.001$). The perinatal mortality rate for cephalic presentations over 2,500 g in the last 5-year period was 1.13 per cent. The perinatal mortality rate for the 124 deliveries of breech presentations was 4.91 per cent (Table III)

One of the six foetal deaths was due to a lethal malformation (anencephaly). The causes of the other foetal deaths were prolapse of the cord (2), intracranial haemorrhage (1) and unknown (2). The corrected mortality rate is 4.04 per cent (Table III). For cephalic presentations the corrected mortality rate is 1.05 per cent.

340 attempts at external version were performed in 296 patients. Two, three or four attempts were made if version was not successful or if the presentation reverted after a successful version. The results are illustrated in Table IV

In the remaining 68 cases of breech deliveries attempts at version were not performed. 31 patients were admitted to the Department in labour without any antenatal care at the Department. 32 patients had a cephalic presentation at the antenatal clinic six to three weeks before term, but there may be a possibility of incorrect diagnosis since no radiographs were taken. Spontaneous version to a breech presentation is also a possibility. In 5 cases external version was contraindicated. 2 patients had antepartum haemorrhage and in 3 patients elective Caesarean section was planned because of the breech presentation in association with previous classical Caesarean sections (2) and pelvic contraction (1). Previous lower segment Caesarean section was not considered as a contraindication to the performance of external version.

In 3 cases external version was followed by complications and one of these resulted in foetal death

IV gravida, 28 years old. 4 weeks before the expected date of delivery the patient was found t have breech presentation. External version was performed. 3 day after the version foetal movements disappeared and labour

began the next day At this time foetal heart sounds could not be heard. Amniocentesis was performed and the liquor amnii was heavily blood-stained. Because of continuous bleeding a lower segment Caesarean section was performed. The uterus was very hard with subserous haemorrhages (Couvelair-uterus) The infant (weight 4 100 g) was macerated and there was a total abruption of the placenta.

In two cases external version was followed by rupture of the membranes and onset of labour In both cases the infants were delivered as a vertex presentation. The birthweights were 3 200 g and 3,300 g and both had a high Apgar Score.

The foetal mortality rate due to version is in this report 0.38 per cent (1 out of 262 versions)

Conclusion

The present series of single births weighing more than 2,500 g shows that the perinatal mortality rate for delivery of breech presentations is about four times as great as for delivery of cephalic presentations.

By performing prophylactic external cephalic version the incidence of breech delivery among infants weighing more than 2 500 g is lowered from 3 15 per cent to 2.05 per cent although, for different reasons version was not attempted in a great many of the breech presentations

The foetal loss due to external version was 0 38 per cent.

The corrected mortality rate for delivery of a cephalic presentation is 1 05 per cent.

The corrected mortality rate for delivery of a cephalic presentation after external version is 1 43 per cent. The mortality rate for delivery of a breech presentation is about 3 times as great.

The conclusion is that there is a need for prophylactic external cephalic version in order to reduce the foetal loss which would otherwise result from breech delivery

SUMMARY

In a retrospective study infants weighing more than 2,500 g the influence of prophylactic external cephalic version is investi

gated. It is shown that by performing external version the incidence of breech delivery decreases by 1.1 per cent, which is statistically significant.

The corrected perinatal mortality rate for delivery of breech presentations is about 3 times as great as the mortality rate for external version followed by delivery of a cephalic presentation.

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OSTEOGENESIS IMPERFECTA

A Report of a Case of the Congenital form

BY

JOHANNES E. BOCK

Osteogenesis imperfecta is a rare disorder characterized by abnormal fragility of the bones resulting in fractures after minimal trauma, blue sclerotics because of thinness of the fibrous tissue deafness because of otosclerosis hyperlaxity of the ligaments, and deficient formation of dental enamel.

In 1835 *Lobstein* described the disease and called it osteopsathyrosis idiopathica (Lobstein s disease) In 1849 *Vrolik* described the congenital form and called it osteogenesis imperfecta. *Looser* (1906) concluded on the basis of histological similarities between Lobstein s and Vroliks disease that they were two forms of the same disturbance although of different severity This is still accepted to-day with only a few modifications (*Seedorff* 1949 *Smars* 1961) The congenital form, osteogenesis imperfecta congenita is usually the more severe with multiple fractures in the newborn or even in utero. The late form, osteogenesis imperfecta tarda is found in infants adolscents, or adults This report concerns the congenital form of the disease.

Little is known about the aetiology Until lately the basic disorder was thought to be a generalized inability of the osteoblasts to make enough lamellar bone (*Rubin* 1964) Recent investigations (*Jett* 1966 *Lee* 1964) employing a quantitative histological measuring technique with tetracycline as a marker seem to show that the only bone disorder is an inability to lay down periosteal bone The result of this disturbance in bone-formation is an irregular bone tissue with the Haversian systems more or less missing The lamellae of the compact bone and trabeculae of the spongy bone are also poorly developed. The

bones are, therefore brittle and have a very thin cortex. The diaphysis of the long bones is deficient and slender while growth and differentiation of the epiphysis remains normal. The disorder involves bones of cartilaginous as well as membranous origin. The calvarium is very thin and in severe cases it consists of a membranous bag with isolated islands of bone tissue

The essential radiological findings in children with osteogenesis imperfecta congenita are a thin bony cortex and a relatively large medullary cavity (*Sarma* 1960) The long bones have a slender shaft widening abruptly towards the epiphysis. Fractures can be seen in any part of the skeleton. They tend to heal with normal speed and often plenty of callus is found at the fracture site The skull shows a mosaic appearance due to the presence of numerous islands of bone tissue Skull fractures are common too The vertebral column is often deformed (kyphosis or scoliosis) because of compression fractures and protrusion of the discs into the vertebral bodies. The disease is well advanced before birth and may be identified on prenatal X-ray films (*Posner Goldman*, 1957)

Genetic studies of the disease (*Seedorff* 1949 *Smårs* 1961 *Sarma* 1960) show that the tarda variety is hereditary with dominant autosomal transmission. However there are different points of view about the congenital variety Of *Seedorff's* (1949) 180 cases there are only 7 cases of the congenital type. In 6 of these cases no signs of the disease were found in the parents. In the seventh case the mother suffered from osteogenesis imperfecta tarda. *Seedorff* (1949) found 6 cases in literature in which a mother with osteogenesis imperfecta tarda gave birth to an infant affected by osteogenesis imperfecta congenita and he used these 6 cases and his own case to show that the congenital type is hereditary too He believes that osteogenesis imperfecta congenita arising from normal parents is a product of a dominant mutation of certain genes. Others believe that osteogenesis imperfecta congenita is carried by a recessive gene (*Gates* 1946) or is not inherited (*Funk* 1940)

Biochemical investigations reveal normal serum calcium and phosphorus levels. The alkaline phosphatase is raised in some cases, but not in all (*Sharma* 1965)

[illegible] Acta [illegible] et gynec. scandinav 48 222, 1969

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Fig. 2.



Fig. 3





Fig. 1

The incidence of the congenital form is not known. *Posner* and *Goldman* (1957) state that osteogenesis imperfecta congenita is seen only once in 40 000 deliveries. *Freda et al* (1961) collected 90 cases from the literature They say 1 in 21 000 Out of *Seedorff*'s (1949) 180 cases only 7 were of the congenital type. *Campbell* (1966) has seen only one case in 15 000 deliveries.

Case Report

AKK (journ. 1063/67–8 Obst.) was a 34-year old woman gravida I para I with a 13 year history of infertility In 1966 she had an operation for a toxic tyroid adenoma The family history showed no evidence of bone diseases. Her last menstrual period was at the beginning of February 1967

Fig. 2.

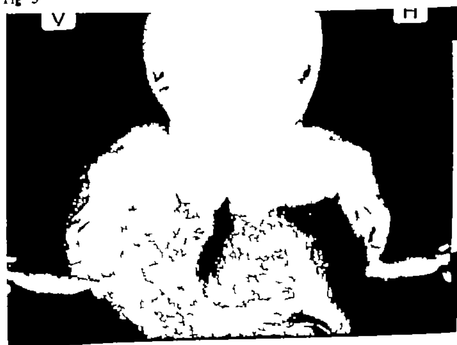
Fig. 3.





Fig 4

Fig 5



Four weeks prior to the expected date of confinement abdominal and vaginal examination revealed breech presentation. External version without anaesthesia was attempted and two days later a cephalic presentation was found. On October 28, 1967 the patient was admitted in early labour. Examination again revealed a breech presentation. Because of the age and infertility of the patient and the breech presentation Caesarian section was performed. The puerperium was normal except for an urticadal rash.

Examination of the baby showed.

Weight 2500 g, length 42 cm. Respiration began within half a minute and there was no cyanosis. The infant developed an increasing respiratory rate (90) with paradoxical respiratory movements of the left part of the chest. The head appeared to be slightly enlarged. The calvarium was very soft and on palpation multiple bone islands could be felt. The eyes were slightly prominent with blue discolouration of both sclerotics. Both upper and lower limbs were deformed with crepitation in many places. The limbs were rather short (Fig 1)

The radiological findings were

Fig 2 A slightly hydrocephalic skull with mosaic appearance but no sign of fractur

Fig 3 X-ray films of the left arm showed a recent fracture of the shaft of the humerus and fractur of the adius and ulna with abundant callus.

Fig 4 An X-ray film of the right arm showed fractures at the upper and lower ends of the shaft of the humerus and fractures of both radius and ulna at the middle of the shaft.

Fig 5 An X ray film of the legs showed fractures of both the femur and the tibia on both sides Diffuse halisteresis was present too.

The baby died when 8 days old

A macroscopic examination showed very brittle bones with multiple fractures recent and old Both sclerotics wer thin and blue. No other malformations were present A microscopic examination showed extremely defective ossification both in cartilage and membranous bones Only few Haversian systems were present in the compact layer and the spongy bone appeared cystic in character The calvarium was thin membrane of connective tissue with irregular osseous plates The sternum was found consisting only of hyaline cartilage without ossification (T Lund, Aarhus)

Comment

This is a classical case of the congenital form of osteogenesis imperfecta. The parents were normal and the disease may be the result of a mutation. Four weeks before term external version of a breech presentation was performed. This version may per



Fig 4

Fig 5



Öckerman, P. A. Acta obst. et gynec. scandinav. 48, 229, 1969
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GLUCOSE-6-PHOSPHATASE IN HUMAN ENDOMETRIUM

BY

P. A. ÖCKERMAN

Glucose-6-phosphatase is an enzyme tightly bound to the endoplasmatic reticulum (microsomes after homogenization and centrifugation) (*Hers* and *De Duve* 1950).

In man it is active in liver, kidneys (*Cori* and *Cori* 1952) and jejunal mucosa (*Öckerman* 1964 and 1965). Although the existence of glucose-6-phosphatase has been suggested in several other tissues there is no convincing evidence of the presence of a specific microsomal enzyme in tissues other than liver, kidneys and jejunal mucosa. Among the tissues studied is endometrium. *Hughes et al.* (1963) speculated on the function of glucose-6-phosphatase in human endometrium, and found a cyclic variation of the activity with the functional state of the tissue. However these authors only measured total glucose-6-phosphate splitting activity without characterizing the activity. Such an assay is totally unsatisfactory as a specific measure of glucose-6-phosphatase activity because other phosphatases, e.g. acid and alkaline phosphatases may have a considerable action on glucose-6-phosphate at pH 6.0–6.5, even in the absence of specific glucose-6-phosphatase (*De Duve et al.* 1949, *Öckerman* 1965).

A highly sensitive method for the assay of glucose-6-phosphatase was recently presented (*Öckerman* 1967). This method greatly facilitated the study of phosphatase activities in human endometrium. The results of such a study are now presented.

haps have been responsible for some of the fractures. The clinical radiological and histological findings are all characteristic.

SUMMARY

A case of osteogenesis imperfecta congenita is presented.

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After 0 20 40 and 60 min. of incubation 0 1 ml of 10 % trichloroacetic acid and 0.2 ml of water were added. The tubes were centrifuged and analysis was performed on 0.2 ml of the supernatant to which 0.3 ml of water 2.5 ml of malachite green and 0 1 ml of Tween 20 had been added.

Fractionation of subcellular particles

Fresh, unfrozen tissues were weighed, homogenized as described above and centrifuged in a Spinco Model L ultracentrifuge (Beckman-Spinco, Fullerton) in a swing-out rotor SW 39 L. The following fractions were collected fraction N (625 g min unbroken cells and nuclei) M (30,000 g min mitochondrial fraction, L (250,000 g min lysosomal fraction) Mc (2,160 000 g min microsomal fraction) and S (Supernatant after 2 160 000 g min) The g minutes during acceleration and deceleration are included in the figures given. The system was devised (P A. Ockerman, unpublished studies) for liver tissue and gave a distinct separation of acid phosphatase (as a marker for lysosomes) from specific glucose-6-phosphatase (as a marker for microsomes) The calculation of the results was as follows The total activity of each enzyme was calculated for each fraction and expressed as a percentage of the activity of the enzyme in question in the whole homogenate The same calculation was made for the protein content in each fraction and the value for the percentage enzyme activity was divided by the value for the percentage protein content. The figure thus obtained was called relative specific activity

Aggregation at pH 5

To aggregate and concentrate microsomes the technique described by *De Duve et al.* (1949) was used. This technique involved incubation at pH 5.0 and 0 C for 15 min, centrifugation and suspension of the sediment in 0.25 M sucrose in 0 001 M EDTA (pH 7 0)

pH-dependency

The buffers used to study the enzyme activities as a function of pH for each experiment are described in the figures. The pH of

Material and Methods

Chemicals

These were as described earlier (*Öckerman* 1967)

Patients

Endometrium was obtained from women aged 22–45 subjected to curettage usually because of menstrual irregularities. No patient with cancer of the endometrium was included. The day of the cycle was variable

Preparation of the tissue

The curettings were immediately blotted on gauze to remove blood and then frozen on solid CO_2 Tissues to be used for centrifugal separation of subcellular particles were chilled in ice and centrifugation was started within one hour The tissue for chemical analysis was rapidly weighed and homogenized in 9 to 99 volumes of 0 25 M sucrose in 0 001 M EDTA (pH 7 0) in an all glass Potter Elvehjem homogenizer The tissue was chilled in an ice bath throughout the procedure.

Enzyme assays

Glucose-6 phosphatase was measured as described earlier (*Öckerman* 1967) using both glucose-6 phosphate (G6P) and β-glycerophosphate (βGLP) as substrates Incubation times were 0 60 90 and 120 min The final volume read in the photometer was 3 1 ml. The difference between the liberation of phosphate from G6P and that from βGLP was used to calculate the activity of glucose-6-phosphatase This must not be confused with total G6P splitting activity

Acid phosphatase was measured in the following way using the same assay principle as for glucose-6-phosphatase 0 02 ml samples of homogenate usually prepared in 0 1 % Triton X 100 0 01 ml of 0 4 M acetate buffer (pH 5 0) and 0 02 ml of 0 5 M βGLP were incubated together at 37 in 0 4 ml stoppered plastic tubes (Beckman Spinco Fullerton) within larger centrifuge tubes as earlier described for glucose-6-phosphatase (*Öckerman* 1967)

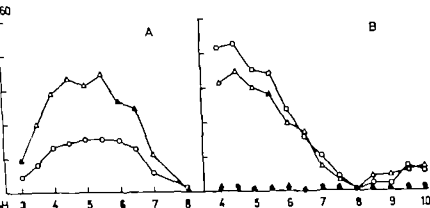


Fig. 1 Effect of pH on phosphatase activities in whole tissue homogenates. *A* Case 3 (Tab. I) Buffers: acetate (pH 3.0–5.5) imidazol (pH 6.0–8.0) Substrates, O—O G6P Δ—Δ βGLP Tissue homogenized 1/100 in 0.25 M sucrose 0.001 M EDTA (pH 7.0) Incubation time 2 hours. Zero minute values deducted. *B*. Case 8 (Tab. I) Buffers: acetate-borate-cacodylate according to *de Duve et al.* (1949). Substrates as in A. ●—● Tissue boiled for three minutes before assay G6P as substrate. ▲—▲ Boiled tissue, βGLP as substrate. Tissue homogenized 1/25 in 0.25 m sucrose 0.001 M EDTA (pH 7.0) with 0.1 % Triton X 100 Incubation time 1 hour Zero minute values deducted

case with higher activity on G6P (Fig. 1) No difference between the pH-curves for the two substrates was apparent in either case The maximum activity on both substrates was noted at pH 4.5–5.0 The non-enzymatic liberation of phosphorus was negligible at all pH levels as evidenced from the experiment with boiled tissue homogenate

Effect of pH on aggregated tissue fractions

Tissue homogenates were aggregated at pH 5.0 and 0 C and the sediment was suspended in sucrose-EDTA. This treatment diminished the phosphatase activity on βGLP more than that on G6P The resulting pH-curves (Fig. 2) indicated the existence of glucose-6-phosphatase activity at optimum pH 5.5–6.5 This was

Table I *Phosphate-Liberating Activity in Human Endometrium on Glucose-6-Phosphate (G6P) and β-Glycerophosphate (βGLP) All Values in μmole P/g Wet Weight/Min*

	Substrate Used		Difference
	G6P	βGLP	G6P βGLP
1	1 03	1.53	−0.50
2.	1.97	4 67	−2.70
3.	0.83	1 48	−0.65
4	0 73	2.85	−2.12
5	1.51	0.53	+0.98
6	0.97	0 48	+0 49
7	0.86	0.52	+0.34
8.	0.83	0 75	+0 08
9	0 75	0 46	+0.29
10	0.80	0.50	+0.30
11	0 68	0.25	+0 43

the incubation mixture was measured to ascertain that no deviations from the expected pH occurred.

Protein assay

This was performed according to *Lowry et al* (1951)

Results

Normal range of phosphatase activities

The phosphate-liberating activity at pH 6 5 on G6P and βGLP of human endometrium is shown in Table I In some cases βGLP was split at a higher rate than G6P In these cases there existed no glucose-6-phosphatase activity when calculated as described previously and a negative value was obtained for the difference G6P splitting minus βGLP splitting activity In other cases a positive value was obtained and thus the existence of a glucose-6 phosphatase as defined in this communication was indicated.

Effect of pH on whole tissue phosphatases

The effect of pH on the liberation of phosphate was studied in one case with higher activity on βGLP than on G6P and in one

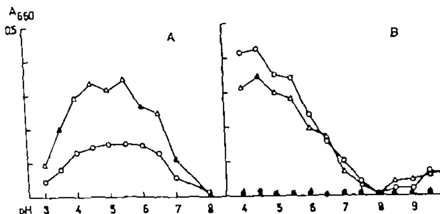


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2	1.97	4.67	−2.70
3	0.83	1.48	−0.65
4	0.73	2.85	−2.12
5	1.51	0.53	+0.98
6	0.97	0.48	+0.49
7	0.86	0.52	+0.34
8	0.83	0.75	+0.08
9	0.75	0.46	+0.29
10	0.80	0.50	+0.30
11	0.68	0.25	+0.43

the incubation mixture was measured to ascertain that no deviations from the expected pH occurred.

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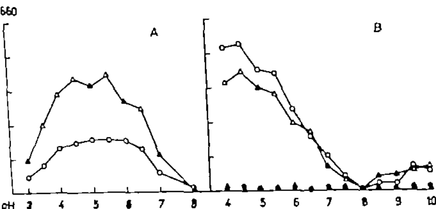


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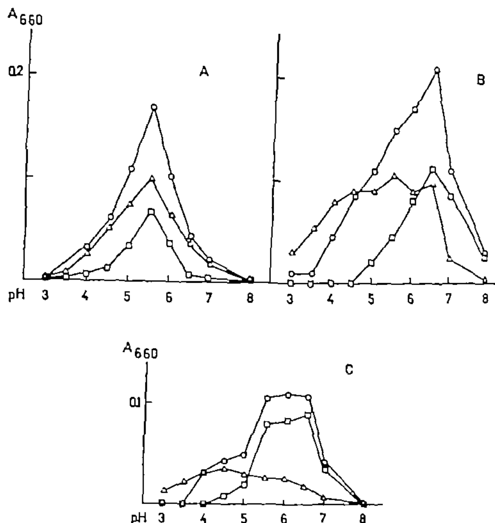


Fig. 2 Effect of pH on phosphatase activities in aggregated fraction of homogenate (see METHODS) *A*. Case 4 Substrates: O—O G6P Δ Δ βGLP □—□ phosphate liberation from G6P minus that from βGLP Homogenization buffers and incubation time as in Fig. 1A. Zero minute values deducted. B. Case 9 Legend as in A. C Case 6 Legend as in A.

true both for Case 4 in whom direct analysis on whole tissue homogenate showed no glucose-6-phosphatase (G6P βGLP) activity (Table I) and also for Cases 6 and 9 in whom such activity was noted in whole tissue homogenates.

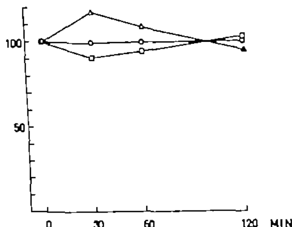


Fig. 3 Stability of phosphatase activities at 37° C in an aqueous homogenate. Tissue homogenate from Case 6 1/50 in water was incubated at 37° for various times up to 2 hours. Phosphatase assays were subsequently performed as described in METHODS after addition of sucrose-EDTA.
○—○ phosphate liberation from G6P
△—△ phosphate liberation from βGLP
□—□ glucose-6-phosphatase (G6P βGLP)

Stability of phosphatase activities at 37 C

When a tissue homogenate in water was treated at pH 5 and 37 C for 10 min the phosphatase activity on both G6P and βGLP was stable (Table II). Stability was also noted for the phosphatase activities in a homogenate in water incubated at 37 C for various times up to 2 hours (Fig. 3).

Localization of phosphatases in subcellular particles

The fractionation of subcellular particles in the ultracentrifuge appeared to be a further means of partly separating the phosphatase activities on G6P and βGLP. In the experimental results recorded in Fig. 4 A it is seen that the relative specific activity

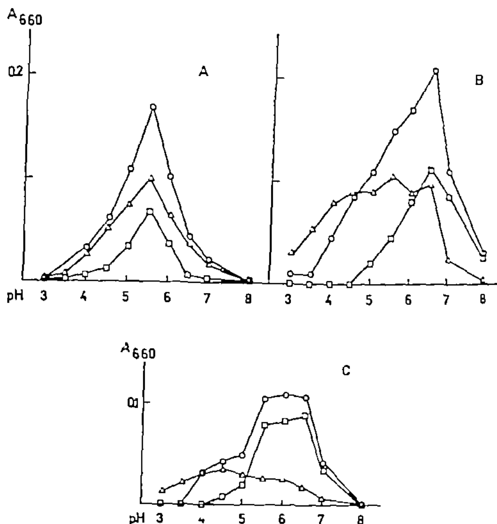


Fig. 2. Effect of pH on phosphatase activities in aggregated fraction of homogenate (see METHODS). A. Case 4. Substrates: O—O G6P △ △ βGLP □—□ phosphate liberation from G6P minus that from βGLP. Homogenization, buffers and incubation time as in Fig. 1A. Zero minute values deducted. B. Case 9. Legend as in A. C. Case 6. Legend as in A.

true both for Case 4 in whom direct analysis on whole tissue homogenate showed no glucose-6-phosphatase (G6P-βGLP) activity (Table I) and also for Cases 6 and 9 in whom such activity was noted in whole tissue homogenates.

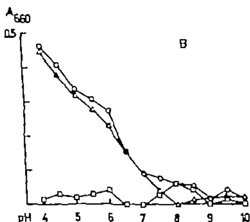


Fig. 4 B: Effect of pH on phosphatase activities in the lysosomal fraction. Buffers as in Fig. 1 B.
○—○ phosphate liberation from G6P
△—△ phosphate liberation from βGLP
□—□ glucose-6-phosphatase (G6P βGLP)

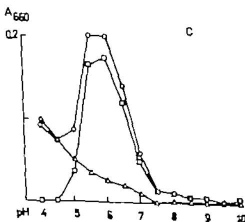


Fig. 4 C. The same experiment on the microsomal fraction.

Table II *Stability of Phosphatase Activities at pH 5 and 37°C*

Tissue homogenate from Case 9 1/10 in water was titrated to pH 5 with HCl and incubated for 10 min at 37 C Phosphatase activities were measured at pH 6.5 Before and After this Incubation Incubation Times 0 10 20 30 min All values in μmole P/g wet weight/min.

Substrate	Direct Assay	Assay after Treatment at pH 5 and 37°C
G6P	0 75	0 78
βGLP	0 46	0 48

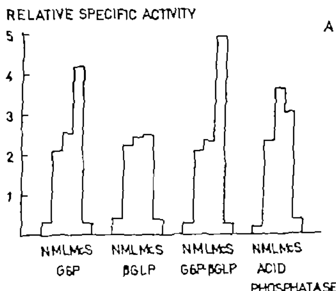


Fig. 4 Subcellular localization of phosphatase activities Unfrozen tissue homogenate from Case 11 1 25 in 0 25 M sucrose in 0 001 M EDTA (pH 7 0) was fractionated in the ultracentrifuge as described in METHODS The sediments were suspended in sucrose-EDTA before the assays In the assay of acid phosphatase Triton X 100 was added to a concentration of 0 1 % (v/v) N = nuclear fraction M = mitochondrial fraction L = lysosomal fraction. Mc = microsomal fraction. S = supernatant

Fig. 4 A. Relative specific activity in each fraction calculated as described in METHODS

trium. Clearly no method of assay of this enzyme can be devised from these findings. Indeed, it is not clear whether any specific glucose-6-phosphatase activity exists even when the splitting at pH 6.5 is greater for G6P than for βGLP

Effect of pH on aggregated tissue phosphatases

The technique used to aggregate microsomes is not specific for these particles, but has been used earlier with success by the author (*Ockerman* 1965) to separate and purify glucose-6-phosphatase from alkaline phosphatase in intestinal mucosa. Although it seemed from the pH-curves from whole tissue homogenate that acid phosphatase was the principal phosphatase in endometrium, the aggregation technique was tried. The results clearly indicate that glucose-6-phosphatase is present in human endometrium provided this enzyme is defined as described previously in this paper The effect of pH on the glucose-6-phosphatase (G6P βGLP) activity clearly differs from that on the βGLP splitting activity and also from that on the total G6P-splitting activity in whole tissue homogenates. These findings may be interpreted as indicating the existence of a specific glucose-6-phosphatase which may be similar to that in liver kidneys and jejunal mucosa. An alternate explanation might be a different pH-dependency of acid phosphatase according to whether G6P or βGLP is used as a substrate

Stability of phosphatases activities at 37 C

Glucose-6-phosphatase in liver kidneys and jejunal mucosa is very labile at 37 C when incubated at pH 5 or without substrate (*De Duve et al* 1949 *Ockerman* 1965) Therefore, the results found here for endometrial glucose-6-phosphatase, indicating a great stability of the total activity on G6P are unexpected, if any specific glucose-6-phosphatase exists in endometrium. At present no explanation for this apparent stability can be given.

Localization of phosphatases in subcellular particles

The results presented in this communication support the concept that glucose-6-phosphatase (G6P βGLP) activity has a different

of glucose-6-phosphatase (G6P βGLP) was maximal in the microsomal fraction. The βGLP splitting activity and acid phosphatase showed a different pattern with maximum activity in both the lysosomal and the microsomal fractions.

The effect of pH on phosphatase activities further illustrated this difference (Fig. 4 B and C), the glucose-6-phosphatase (G6P βGLP) activity being optimum at pH 6.0 in the microsomal fraction. The phosphatase activity on βGLP, however, was greater at more acid pH levels. In the lysosomal fraction much less glucose-6-phosphatase (G6P βGLP) activity was found with no measurable pH-optimum, while the β-glycerophosphatase activity was most active at pH 4 or possibly even lower.

Discussion

Normal range of phosphatase activities

No normal values for the activity of glucose-6-phosphatase in endometrium can be stated. This is because the activity on βGLP was often higher than that on G6P. If any specific glucose-6-phosphatase activity existed in these cases it would be concealed by the higher activity of other phosphatases on βGLP. Thus the method used for the assay and calculation of specific glucose-6-phosphatase may not give a true estimation of the enzyme activity if this is low compared with that of other phosphatases present. However, no other method, except possibly the use of inhibitors (*Di Bella et al.* 1963) is at present available to measure specific glucose-6-phosphatase in the presence of other phosphatases. An attempt to use such inhibitors was unsuccessful, however (P. A. Öckerman, unpublished experiments).

The results very clearly demonstrate that it is inadequate to measure the total phosphate liberating activity on G6P and, as has been the practise, call this glucose-6-phosphatase (*Hughes et al.* 1963). A large part of the activity thus measured comes from other phosphatases.

Effect of pH on whole tissue phosphatases

The pH curves on whole tissue homogenates further demonstrate the difficulty in measuring glucose-6-phosphatase in endome-

trium. Clearly no method of assay of this enzyme can be devised from these findings. Indeed, it is not clear whether any specific glucose-6-phosphatase activity exists, even when the splitting at pH 6.5 is greater for G6P than for βGLP

Effect of pH on aggregated tissue phosphatases

The technique used to aggregate microsomes is not specific for these particles but has been used earlier with success by the author (*Ockerman* 1965) to separate and purify glucose-6-phosphatase from alkaline phosphatase in intestinal mucosa. Although it seemed from the pH-curves from whole tissue homogenate that acid phosphatase was the principal phosphatase in endometrium, the aggregation technique was tried. The results clearly indicate that glucose-6-phosphatase is present in human endometrium provided this enzyme is defined as described previously in this paper. The effect of pH on the glucose-6-phosphatase (G6P βGLP) activity clearly differs from that on the βGLP splitting activity and also from that on the total G6P-splitting activity in whole tissue homogenates. These findings may be interpreted as indicating the existence of a specific glucose-6-phosphatase which may be similar to that in liver kidneys and jejunal mucosa. An alternate explanation might be a different pH-dependency of acid phosphatase according to whether G6P or βGLP is used as a substrate

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Localization of phosphatases in subcellular particles

The results presented in this communication support the concept that glucose-6-phosphatase (G6P βGLP) activity has a different

subcellular localization from that of acid phosphatase and the activity on βGLP at pH 6 5 The fact that the patterns of the latter two activities were very similar might indicate that the βGLP splitting activity at pH 6.5 reflects mainly acid phosphatase activity

The pH-dependency of the activities measured in the lysosomal and microsomal fractions further support the idea that there exists in endometrium a glucose-6-phosphatase activity not identical with acid phosphatase The fact that the relative specific activity of acid phosphatase was the same in both the lysosomal and the microsomal fractions indicates either contamination by lysosomes in the microsomal fraction or that the microsomes contain an enzyme active on βGLP at pH 4 as well as at pH 6.5 This enzyme may or may not be the glucose-6-phosphatase discussed here

The results of the assays on subcellular particles give evidence against the alternative explanation put forward in the discussion of the aggregation experiments Thus the different pH-curves found on aggregated material using G6P and βGLP respectively could not be caused by the same enzyme

General Conclusions

All the findings support the theory that there is in human endometrium a glucose-6-phosphatase which differs from acid phosphatase This enzyme might have the same intracellular localization as the specific glucose-6-phosphatase in liver although there is no direct proof of this The enzyme is more stable than the specific glucose-6-phosphatase in liver kidneys and jejunal mucosa It may be genetically the same enzyme as glucose-6-phosphatase in the other tissues but its properties are not identical. One means of elucidating the possible genetic identity of glucose-6-phosphatase in endometrium and liver kidneys and jejunal mucosa would be by investigation of patients with glycogen storage disease type I who have a genetically determined deficiency of glucose-6-phosphatase in liver kidney and jejunal mucosa (*Cori* and *Cori* 1952 *Ockerman* 1964) So far it has not been possible to obtain endometrium from such a patient.

SUMMARY

Phosphatase activity in human endometrium was studied using glucose-6-phosphate (G6P) and β-glycerophosphate (βGLP) as substrates. When whole tissue homogenates were used the splitting of G6P was higher than that of βGLP in some patients. By studying the pH-dependency of the activities on G6P and βGLP no definite indication of the existence of a specific glucose-6-phosphatase was obtained.

By aggregating microsomes at pH 5 0 at 0 C and by ultra centrifugal fractionation of subcellular particles and assay of G6P and βGLP-splitting activities at various pH levels results indicated the existence, in the microsomal fraction, of a glucose-6-phosphatase not identical with acid phosphatase. This enzyme had its optimum activity at pH 5.5–6.5 and was stable at 37 C even at pH 5 It thus resembles in many but not all, respects the specific glucose-6-phosphatase known to exist in liver kidney and jejunal mucosa.

Acknowledgements

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LIVER REACTION IN CONNECTION WITH ORAL CONTRACEPTIVE STEROIDS

BY

JOHAN BROHULT AND ANDERS WESTGREN

Many retrospective studies have shown that there is probably a causal connection between icterus and the use of peroral contraceptive steroids (*Baines* 1965 *Larsson-Cohn* 1965 *Thulin* and *Nermark* 1966) One of the purposes of this prospective study was to investigate how often the introduction of peroral contraceptives is followed by clinically important disturbances in the liver

Rises in serum transaminases (*Knutson et al* 1965 *Larsson Cohn* 1966 *Orellana Alcalde* and *Dominguez* 1966) and in serum ornithine carbamoyl transferase (S OCT) (*Brohult* and *Westgren* 1965) have been observed after contraceptive steroids and an increase in bromsulphthalein retention has also been demonstrated after peroral contraceptives (*Eisalo et al* 1964 *Larsson-Cohn* 1967) OCT was considered to be a particularly suitable enzyme for this study since it occurs only in the liver and even a minor disturbance in this organ elicits a rise I S-OCT (*Reichard* 1962) Analyses were made of the S-OCT at specific intervals in order to elucidate how the liver reacts after the introduction of contraceptive steroids as well as to investigate whether any liver injury can be detected at an early stage

Material

The study was made on 127 women who applied to the gynecological clinic for peroral contraceptives These women were selected as follows

1. Only women with no liver diseases in the anamnesis were included.
2. Only clinically healthy women with a normal gynecological status were included.
3. All women with an initially pathologically elevated S-OCT (>4 0 mu) and/or S-GPT (>40 units) were excluded. This applied in 7 cases.
4. Women who missed one of the regular blood tests were excluded. This applied to 149 cases the test after one month being missed by 137 women and the test after three months by 12 women. Since so many women missed the test after one month unfortunately it became practically impossible to remind them all by letter or by telephone. Such reminders, were, however sent out each time someone missed one of the blood tests after 3 6 or 12 months.

The contraceptive tablets were prescribed in the ordinary manner the preparations being the four most commonly used in Sweden 1965 and 1966 ethinyloestradiol 0 05 mg, norethisterone 4 mg (Anovlar®) mestranol 0 1 mg, norethisterone 2 mg (Conluten®) mestranol 0.075 mg, norethynodrel 5 mg (Enavid Mite®) and mestranol 0.075 mg, lynestrenol 2.5 mg (Lyndiol Mite®) Since no differences were found between these four groups they have been combined in the following account.

Having received their prescriptions, the women were sent to have a sample of blood taken at the laboratory the plan being to take further samples in 1 3 6 and 12 months time.

Of the 127 women included in the study 18 stopped using the preparation after about 3 months and 7 stopped after about 6 months. Of these 25 women who stopped using the preparations (*cf* Tables III and IV) 19 did so because of side effects (*cf* Fig. 4) and 6 for fear of side effects or because they wished to become pregnant

Data concerning the 102 women who were followed throughout the study are given in Tables I II and Figs. 1–3 Their ages when treatment started varied between 18 and 47 years. These 102 women included seven who developed side effects towards the end of the year but who nevertheless agreed to continue with

the preparation for just under 12 months Data on these seven women are given in Fig 4 together with the 19 women who stopped because of side effects after 3–6 months.

Methods

Sampling

Samples for the analyses of serum enzymes were taken on five occasions before the introduction of the contraceptive steroids, after 1 month of medication (±1 week) after 3 months (±2 weeks) after 6 months (±3 weeks) and after 12 months (±6 weeks)

Analyses

Recognized methods were used for the statistical calculations (*Dixon* and *Massey* 1957)

By reproducibility (coefficient of variation) is meant the error of the method calculated from duplicated determinations in per cent of the mean of the duplicate determinations

$$\frac{100\sqrt{\frac{\Sigma d^2}{2n}}}{\bar{x}}$$

The OCT activity was determined by incubation of serum with citrulline carbamoyl ^{14}C in arsenate buffer (*Reichard* 1964) The results are expressed in nanomoles (nm) $^{14}CO_2$ liberated by 0.5 ml serum during two hours incubation under standard conditions Normal value ≤4 nm (*Beckman et al* 1966) i e 0 004 micromoles $^{14}CO_2$ Reproducibility 8 per cent.

GOT and GPT were determined by the NADH method (*Karmen* 1955) as modified by *Ordell* (1956) Normal value <40 Karmen units (*Allgén* 1962) Reproducibility 5 per cent.

Lactic dehydrogenase (LD) was determined by the method of *Wróblewski* and *LaDue* (1955) Normal value 100–300 Wróblewski units. One unit corresponds to a consumption of 0.53 micromoles per litre and minute Reproducibility 4 per cent.

Bilirubin was determined by the method of *Jendrassik* and *Gröf* (1938) according to the modification of *Nosslin* (1960) Normal value. < 1.2 mg per 100 ml (*Nosslin* 1960) Reproducibility 5 per cent.

The alkaline phosphatase activity in serum was determined by the method of *Roos* (1963) according to the modification of Kabi. Normal value 2–8 Buch & Buch units (*Buch* and *Buch* 1939) Reproducibility 6 per cent.

The thymol turbidity was determined according to *MacLagan* (1944) Normal value < 4 MacLagan units. Reproducibility 4 per cent.

Results

The 102 women who completed the study displayed a slight rise in the alkaline phosphatase activity in serum after one month ($P < 0.05$) On no occasion were any significant changes detected in bilirubin, thymol turbidity or LD in serum (*cf* Table II) Two women who stopped taking the tablets because of icterus and itching (cases 93 and 108 *cf* Fig. 4) displayed moderately elevated serum bilirubin (3.2 and 4.7 mg per cent respectively)

In the 102 women who completed the study S-OCT (*cf* Table I A-C) S-GOT (*cf* Fig. 2) and S-GPT (*cf* Fig 3) showed a significant rise after one month ($P < 0.001$) and then successively dropped again (*cf* Fig. 1–3) The average levels of S-OCT after 1 month (4.8 nm) 3 months (3.7 nm) 6 months (3.3 nm) and 12 months (3.0 nm) are all significantly higher than the initial level (1.7 nm) with $P < 0.001$ 0.001 0.01 and 0.05 respectively

As will be seen from Fig. 1 the subjects were divided into three groups according to the course of S-OCT group A (plain group) comprises those women whose serum-OCT did not exceed 4.0 nanomoles (nm) in any of the tests (Table I A) group B (hill group) comprises the women whose S-OCT exceeded 4.0 nm on one or several occasions but was found to have normalized (4.0 nm or below) after 12 months (Table I B) group C (rise group) finally comprises the women whose S-OCT exceeded 4.0 nm after 12 months (Table I C) The rises in S-OCT after 1 and 3 months are statistically significant in all

Table I A. *The 47 Women out of 102 with S-OCT Never Exceeding 4.0 Nanomoles*

Case	Initial 0	Months after Start			
		1	3	6	12
5	0.1	0.6	1.2	0.0	0.4
6	0.7	1.0	1.6	1.0	0.9
7	0.7	0.6	2.2	1.2	1.3
8	0.5	2.0	1.3	0.0	0.2
11	0.8	1.1	2.0	1.5	0.9
15	1.2	2.8	2.3	1.9	1.3
16	1.0	2.5	2.8	3.6	2.4
18	3.3	3.8	2.2	2.2	1.4
20	2.1	2.9	1.9	1.4	0.4
32	1.5	1.4	2.3	1.7	1.9
35	1.4	4.0	3.4	3.0	1.0
36	0.5	2.4	0.5	1.6	2.0
38	0.7	1.7	2.6	0.4	0.4
39	0.1	2.9	1.3	1.4	3.3
41	2.7	1.4	0.5	1.1	1.9
43	1.3	1.3	0.8	0.5	0.5
48	0.5	2.7	1.0	0.6	0.1
55	0.1	3.4	1.0	1.7	1.7
56	1.0	1.3	1.5	3.5	1.5
57	0.0	0.3	1.6	0.7	0.9
62	1.2	3.4	2.5	0.3	1.4
75	1.0	2.5	1.8	2.0	1.5
78	2.0	1.9	1.7	2.3	0.7
79	0.9	3.9	3.8	3.9	3.3
80	4.0	3.7	2.4	3.8	3.8
86	2.7	2.3	1.8	1.9	2.6
87	1.2	1.7	2.3	2.9	3.8
91	2.7	1.6	1.0	1.1	1.5
95	0.8	1.7	1.8	2.8	1.2
96	0.3	1.3	3.0	1.8	0.3
97	2.9	1.4	3.4	3.9	3.0
98	1.7	2.2	1.1	1.0	1.4
100	0.5	2.9	1.3	1.8	0.3
101	0.3	0.9	0.3	0.6	0.3
104	1.5	1.6	1.0	1.8	1.3
107	0.7	3.1	2.0	1.5	0.2
109	1.4	1.8	2.6	1.5	2.2
112	1.0	1.5	1.3	1.8	0.3
114	1.0	1.5	2.3	1.0	2.3
117	1.3	2.7	1.9	2.1	0.5
125	0.8	2.1	0.8	0.3	1.0
126	0.3	0.6	1.8	0.6	1.5
129	0.4	3.9	3.0	2.2	1.0
130	1.3	3.9	1.7	1.4	0.2
133	1.2	1.1	2.4	1.1	0.8
137	1.4	3.0	1.6	0.7	1.3
139	1.8	2.7	1.9	2.0	1.2
$\bar{x}$	1.2	2.1	1.8	1.6	1.3
SD	0.9	1.0	0.8	1.0	1.0
Range	0.0–4.0	0.3–4.0	0.3–3.8	0.0–3.9	0.1–3.8

Table I B *The 37 Women out of 102 with S-OCT Exceeding 4.0 Nanomoles (nm) on One or Several Occasions but with S-OCT below 4 0 nm after One Year*

Case	Initial 0	Months after Start 1	3	6	12
3	1.6	4 1	3.5	1.8	1.2
4	1.6	4.7	0 8	1.2	1.0
10	3 4	9.7	7.7	6.2	3.7
19	2 5	2.7	3.2	4.9	3.2
21	1 3	12.0	7 1	4 1	3.6
22	2.4	4 4	3.9	2.3	1.9
23	2 0	4.2	2.8	1 9	3 1
27	1 9	16.3	2.3	4 7	1.8
30	1.8	4 4	3.2	2 6	3.3
34	1 6	9 2	15.2	7.0	3.4
37	0 9	1 1	1.8	5.5	2.5
45	2.4	5.3	7 1	5.9	2 0
49	1 4	5.9	2.5	0.4	1 4
50	1 1	14 6	8.3	4 1	0.3
60	1 8	9 0	0.8	1.6	0.3
67	2 4	5 0	1.8	1.9	1.2
70	1.6	5 2	5.0	1.8	2.8
71	0 7	4.9	2.1	1.8	1.1
74	0 5	4.2	2.7	0 7	1.0
81	2 0	5.3	2.2	0.5	3 8
90	2 4	5.8	3.9	1.3	0 0
94	0 8	3 8	4.5	4 0	3.2
99	0 8	7 4	6.3	1.7	1.7
103	1 1	19.6	1 1	0 7	0.2
105	0 6	1.8	0.8	6 7	1 1
111	1.5	8.0	6.0	2 9	1.1
113	0 3	8 9	5.5	2.0	3 6
115	2 6	17 5	2.6	1 7	2 7
120	2 8	5 0	2 7	3.7	3 1
122	2 2	2.3	2 4	6.5	1.6
123	2 1	8.8	2 7	1.0	1.2
127	3 0	15 7	14.0	6.2	3 9
128	1.3	10.5	5.6	1.6	0.2
134	3 4	7 3	6.0	1 4	2.2
141	0 9	3.2	5 8	3.3	1.8
142	3 1	1.5	8 0	0 0	2.4
143	3.1	5 7	1 7	1.0	2.5
$\bar{x}$	1 8	7 2	4 4	2.9	2 0
SD	0 9	4 6	3 3	2.1	1.2
Range	0 3–3 4	1 1 19 6	0.8–15.2	0 0–7.0	0 0–3 9

Table I C. *The 18 women out of 102 with S-OCT Exceeding 4.0 Nanomoles after One Year*

Case	Initial 0	Months after Start 1	3	6	12
12	0.9	5.8	6.2	4.5	7.2
17	2.8	7.4	9.5	6.6	9.8
23	2.5	2.3	2.7	38.9	24.5
25	1.3	5.6	8.8	10.2	13.6
33	3.8	7.5	5.5	5.1	7.1
44	3.5	9.1	8.0	8.8	10.7
46	4.0	10.3	7.3	3.0	6.2
52	0.8	3.8	3.2	2.1	4.3
58	3.8	9.4	4.6	6.7	11.3
63	2.6	8.9	12.1	9.6	16.3
69	2.5	10.1	18.0	14.3	16.5
73	3.7	5.4	14.6	1.9	4.7
83	2.8	3.6	4.0	7.6	6.1
84	1.0	12.4	3.2	5.4	5.2
105	3.9	7.7	9.8	18.0	8.5
116	4.0	1.1	1.9	4.3	4.2
132	1.2	5.9	7.8	4.5	6.8
135	2.0	4.2	4.1	2.6	5.3
$\bar{x}$	2.6	6.7	7.0	8.6	9.4
SD	1.2	3.0	4.4	8.7	5.4
Range	0.8–4.0	1.1–12.4	1.9–18.0	1.9–38.9	4.2–24.5

three groups ($P<0.001$). After 6 and 12 months however the rise is still significant only in group C ($P<0.01$). The initial level of S-OCT in group C is significantly higher than the initial levels in the other two groups ($P<0.01$).

There were 5 women who stopped taking the tablets because of itching. They all displayed a pathological rise in S-OCT (>4.0 nm) when they stopped.

Side reactions that caused cessation of treatment with oral contraceptives after 3–12 months were observed in 26 of the 127 women (see Fig. 4).

An attempt was made to follow up the 149 women who missed one of the tests but it proved very difficult to obtain answers from them all. From the answers that were obtained, however

Table II. *Mean Serum Levels of* OCT, GOT, GPT, LD, *Bilirubin, Alkaline Phosphatases and Thymol Turbidity in 102 Women Using Oral Contraceptive Steroids for One Year*

Analysis	Initial 0	Months after Start 1	3	6	12
OCT (nanomoles)	1.7	4.8	3.7	3.3	3.0
GOT (Karmen units)	21	25	23	22	22
GPT (Karmen units)	20	32	27	24	23
LD (Wroblewski units)	208	213	205	208	206
Bilirubin (mg/100 ml)	0.47	0.53	0.49	0.52	0.50
Alkaline phosphatases (Buch and Buch units)	3.5	3.8	3.7	3.6	3.5
Thymol turbidity (MacLagan units)	1.0	1.1	1.0	1.1	1.1

It seems that roughly half did not turn up because they had stopped using the contraceptive in question, while the other half either had not wished or were unable to come to the hospital for a blood test. Of those who had stopped using oral contraceptives, roughly half had done so for fear of secondary effects about which they had read in the press while roughly half had done so because of secondary effects such as putting on weight, a feeling of tension in the breasts, breakthrough bleedings or mental complaints such as depression, anxiety and irritability. On the other hand, no serious secondary effects were found among these women. Some of them even had difficulty in remembering, or were unwilling to state why they had stopped using peroral contraceptives partly because the follow-up study was not conducted until one or two years after the contraceptive had been prescribed for the first time.

Table I C. *The 18 women out of 102 with S OCT Exceeding 4.0 Nanomoles after One Year*

Case	Initial 0	Months after Start			
		1	3	6	12
12	0.9	5.8	6.2	4.5	7.2
17	2.8	7 4	9.5	6.6	9 8
23	2.5	2.3	2.7	38.9	24.5
25	1.3	5 6	8.8	10.2	13.6
33	3.8	7.5	5.5	5 1	7 1
44	3.5	9 1	8.0	8.8	10.7
46	4 0	10.3	7.3	3.0	6.2
52	0.8	3.8	3.2	2.1	4.3
58	3.8	9 4	4.6	6.7	11.3
63	2.6	8.9	12.1	9.6	16.3
69	2.5	10 1	18.0	14.3	16.5
73	3 7	5 4	14.6	1.9	4 7
83	2.8	3.6	4.0	7.6	6.1
84	1.0	12.4	3.2	5 4	5.2
105	3.9	7 7	9.8	18.0	8.5
116	4 0	1 1	1.9	4.3	4.2
132	1.2	5.9	7.8	4.5	6.8
135	2.0	4.2	4 1	2.6	5.3
x̄	2.6	6.7	7 0	8.6	9 4
SD	1.2	3.0	4 4	8 7	5 4
Range	0.8–4 0	1 1–12.4	1.9–18.0	1.9–38.9	4.2 –4.5

three groups (P < 0 001) After 6 and 12 months however the rise is still significant only in group C (P < 0 01) The initial level of S-OCT in group C is significantly higher than the initial levels in the other two groups (P < 0 01)

There were 5 women who stopped taking the tablets because of itching They all displayed a pathological rise in S-OCT (> 4 0 nm) when they stopped.

Side reactions that caused cessation of treatment with oral contraceptives after 3–12 months were observed in 26 of the 127 women (see Fig 4)

An attempt was made to follow up the 149 women who missed one of the tests but it proved very difficult to obtain answers from them all. From the answers that were obtained, however

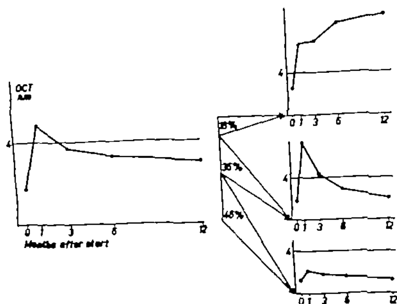


Fig 1 S-OCT in 102 women using oral contraceptive steroids for one year The small figures represent the three groups based upon the course of S-OCT

Table IV *The 7 Women (out of 127) Who Stopped Using Oral Contraceptives after 6 Months*

Case	Initial 0	Months after Start		
		1	3	6
9	3 2	5.2	8 6	7.7
42	2 0	3 3	1.8	2.1
54	1 2	8 0	3.0	0.8
68	0 6	1 5	2.9	0 1
92	2 1	3 2	4 8	3 4
93	1 3	6.0	5.7	5 4
118	2 3	4.5	2 7	3 2
Mean	1.8	4.5	4 2	3 2

Table III *The 18 Women (out of 127) Who Stopped Using Oral Contraceptives after 3 Months*

Case	Initial 0	Months after Start	
		1	3
13	1.2	0.8	1.1
24	1.1	0.1	1.4
29	0.5	11.0	.6
40	1.8	5.9	1.6
51	1.4	3.5	2.9
53	1.4	2.6	8.7
61	0.4	4.5	2.0
64	4.0	4.7	3.3
65	3.1	1.7	1.2
76	3.5	34.1	20.5
88	1.4	0.2	0.6
89	1.0	26.8	17.5
102	1.1	1.3	1.8
108	0.9	1.2	15.3
124	2.3	1.9	1.8
131	3.1	9.6	30.1
136	2.5	13.4	5.5
146	0.6	3.4	1.7
Mean	1.7	7.0	7.0

Discussion

Treatment with contraceptive steroids places an additional load on the excretory function of the liver (*Adlercreutz* 1964, *Eisalo et al.* 1965, *Sherlock* 1968) and induces changes in the metabolism of carbohydrates (*Wynn* and *Doar* 1966), lipids (*Wynn et al.* 1966, *Aurell* and *Cramér* 1966) and proteins (*Robertson* 1967). Many cases of icterus after contraceptive steroids have been reported (e.g. *Cullberg et al.* 1965, *Orellana Alcalde* and *Domingue* 1966) and it is considered that a relationship exists between peroral contraceptives and icterus (*Baines* 1965, *Larsson-Cohn* 1965). One of the aims of the present study was to assess the incidence of clinically relevant effects on the liver. The 5 cases with itching and fatigue (2 of which also displayed icterus) all had high S OCT levels (*cf.* Fig. 4). The symptoms

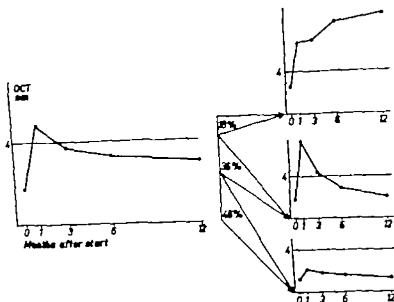


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Table III *The 18 Women (out of 127) Who Stopped Using Oral Contraceptives after 3 Months*

Case	Initial	Months after Start	
	0	1	3
13	1.2	0.8	1 1
24	1 1	0 1	1 4
29	0.5	11 0	7.6
40	1 8	5.9	1 6
51	1 4	3.5	2.9
53	1 4	2.6	8.7
61	0 4	4.5	2.0
64	4 0	4 7	3.8
65	3 1	1 7	1.2
76	3.5	34 1	20.5
88	1 4	0.2	0.6
89	1 0	26.8	17.5
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Discussion

Treatment with contraceptive steroids places an additional load on the excretory function of the liver (*Adlercreut* 1964 *Eisalo et al* 1965 *Sherlock* 1968) and induces changes in the metabolism of carbohydrates (*Wynn* and *Doar* 1966) lipids (*Wynn et al* 1966 *Aurell* and *Cramer* 1966) and proteins (*Robertson* 1967) Many cases of icterus after contraceptive steroids have been reported (*e g Cullberg et al* 1965 *Orellana Alcalde* and *Dominigue* 1966) and it is considered that a relationship exists between peroral contraceptives and icterus (*Baines* 1965 *Larsson Cohn* 1965) One of the aims of the present study was to assess the incidence of clinically relevant effects on the liver The 5 cases with itching and fatigue (2 of which also displayed icterus) all had high S-OCT levels (*cf* Fig 4) The symptoms

group C (rise group) was significantly higher than in either of the other two groups.

The present results differ from those reported in many large studies for instance in the United States (*Swaab* 1964 *Tyler* 1964) The explanation for the higher incidence of clinically relevant liver reactions (4 per cent) is partly that this incidence appears to be greater in Scandinavia than in other parts of the world and partly that women who develop secondary effects stop using oral contraceptives and consequently tend to eliminate themselves from the subjects included in large retrospective studies. The relatively high proportion of transient liver reactions is partly due to the prospective nature of the present study with frequent blood tests and partly to the use of S-OCT analysis which is the most sensitive test available for demonstrating liver reactions.

S-OCT analyses appear to be of practical clinical value in two contexts

1 In the case of women who call for particular caution during treatment with oral contraceptives e.g. after a recent hepatitis or a suspected syndrome of recurrent cholestatic jaundice of pregnancy (Editorial Brit. Med. J 1967 *Sherlock* 1968)
2 In the case of women who during treatment with oral contraceptives display symptoms suggestive of liver disturbances, e g itching.

A normal S-OCT level in these contexts is a strong indication that the oral contraceptives ha e not elicited liver injury

SUMMARY

Out of 127 women who used oral contraceptives for at least 3 months, 5 (4 per cent) developed an itch (2 also developed icterus) that disappeared when the medication was stopped all 5 had pathologically elevated serum OCT Out of 102 women who used oral contraceptives for one year 47 (46 per cent) displayed no or only a slight and transient rise in S-OCT 37 (36 per cent) a pathological but transient rise in S-OCT and 18 (18 per cent) a pathological and successively increasing rise in S-OCT

et al 1955 *Kyle* and *Hess* 1956) Oral contraceptive steroids are reported to result in an increase in lean body mass and a positive nitrogen balance (*Lecocq et al* 1967) and an increased protein synthesis should ultimately lead to an enhanced break down of protein and production of urea. This may be a reason why the women in our study displayed a rise in S-OCT

There is morphological evidence that the synthesis of protein in the liver increases in conjunction with the use of oral contra ceptives. Thus an increase in the free ribosomes arranged in rosettelike and spiral formations has been demonstrated by electronmicroscopy of the human liver after the administration of methandrostenolone (*Orlandi* and *Jezéquel* 1962) and it has been shown that such findings are associated with increased production of proteins

Some of the present women particularly those in group C (rise group) displayed such a pronounced rise in S-OCT that this can hardly be ascribed solely to a corresponding rise in the production of OCT in the liver It seems more probable that these pronunced rises in S-OCT reflect changes in the per meability of the mitochondria. Liver biopsies and electronmicroscopic examination performed on women using oral contra ceptives have disclosed ultra structural changes in the mitochondria (*Orlandi* and *Jézéquel* 1962 *Anberg*, 1966) and it is to these that OCT are mainly located (*Krebs et al* 1955)

The transient rise in S-OCT displayed by nearly all the women in our study thus seems to be ascribable to increased enzyme synthesis and/or mitochondrial changes Once the liver has ad justed to the increased load the synthesis and/or the leakage of enzymes decreases and S-OCT is normalized. The present study also disclosed transient rises in S GOT S-GPT and serum alka line phosphatase This is in good agreement with studies by *Larsson-Cohn* (1967) who reports that in 22 women, the frequency of elevated transaminases in serum was maximum after 1–2 months of therapy and then gradually decreased while the frequency of increased bromsulphalein (BSP) retention reached a maximum after 3 months of therapy and then remained un changed.

It is interesting to note that the mean initial S-OCT level in

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The rises in serum OCT are probably partly due to altered permeability as a result of mitochondrial changes and partly to an increased synthesis of OCT in the liver An analysis of serum OCT seems to be of prognostic value with respect to liver injuries in women who use oral contraceptives

Acknowledgement

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Table I. *Distribution of Patients According to Age and Parity*

Age	Par 0	Para 1 2	Par 3-4	Para 5-6
20	3	1		
20-29	2	10	5	
30-35		5	3	1
> 35	1	10	2	1
Totals	6	26	10	2

One or two days before the operation of conization the patients were subjected to hysterosalpingography essentially in the way described by *Asplund* (1952) in his investigation of the radiological appearance of the cervix and isthmus. In the present series to avoid masking of the cervix by the cone of the instrument the cone was usually cut down. The contrast medium used was viscous perjodal H.

The radiological examination was repeated about four weeks after operation and again 6 to 12 months later using the same contrast medium and the same projections and, when possible on the same day of the menstrual cycle as the primary examination. The pre- and postoperative films were then compared to assess the calibre of the cervix (approximately estimated as small, average, large) the diameter of the internal os (mm in frontal plane) and the pattern of the cervical mucosa (well defined plicae palmatae plicae seen only as a serrated line and plicae not visible) Notes were also made of any abnormalities such as irregular pattern of the mucosal relief pockets, filling of glandular ducts, synechiae and polyps.

Further specimens for histological examination were available from seven women reoperated upon because of a suspected local recurrence within 3 months (1 patient) 7 months (2 patients) 11 13 months (3 patients) and 24 months (1 patient) of the primary operation.

When patients had become pregnant after the operation, and until 1967 details f their histories during pregnancy and the puerperium were obtained from the hospital records.

Kullander Stig and Wehlin Lennart Acta obst. et gynec. scandinav 48, 258, 1969

From the Department of Obstetrics and Gynaecology (Prof S Kullander) and the Roentgendiagnostic Department (Prof S Welin) Malmö General Hospital Malmö Sweden

THE UTERINE CERVIX BEFORE AND AFTER COLD KNIFE CONIZATION

BY

STIG KULLANDER AND LENNART WEHLIN

Cold knife conization is widely practised in the treatment of cytologically suspected cancer of the uterine cervix stage 0 It is stated that this procedure does not interfere with reproduction but it is not known whether the cervix is afterwards anatomically and physiologically normal. Some investigators believe that cervical disorders with infertility abortion and premature births, are more common after conization. Late symptoms resulting from cervical stenosis have been described but not properly investigated. With the increasing frequency of cytological examination of cervical smears and the increasing number of young women subjected to conization of the uterine cervix it is important to ascertain whether any permanent anatomical or physiological sequelae occur after the operation. This problem has been studied on the basis of the patient s history hysterosalpingography and histological examinations.

Material and Methods

The clinical material consisted of 44 women aged 16 to 49 years who were subjected to cold knife conization in 1962 or 1963 The ages and parities of the patients are shown in Table I In 10 of the specimens obtained at operation the histological changes in the epithelium were less than that of fully developed carcinoma *in situ*



1



1b



1

Fig 1 A 37 year old Para II in wh
cancer situ had been radically e
pated Normal pregnancy one year a
operation Cervix before ( ) 3 w
(b) and 10 months after ( ) coniza
Normal findings

Table II. Preoperative Roentgen Appearance of Cervix. (Italics give number of cases w[...] cancer in situ could not be proven histologically)

Phase of Menstrual Cycle	Width of Cervix			Internal Os (mm)	Mucosal Relief			Abnor. Findin[gs]
	Small	Average	Large		No Visible Plicae	Slight Plication	Well Defined Plicae	
Proliferative		29		1–*9* (mean 4.9)	13+*2*	10+*2*	1+*1*	0
Secretory		13		2–8 (mean 4.3)	4+*3*	5	1	0

Results

Radiological findings

Table II gives the preoperative appearance of the cervix in 42 menstruating women grouped according to the menstrual phase at the time of examination (judged from the histological appear ance of the endometrium at operation) and according to whether the histological diagnosis was cancer *in situ* or only slight atypical epithelial proliferation. The table shows that the width of the cervix the diameter of the internal os and the mucosal pattern were normal before operation. Hence the radiological examination did not allow a diagnosis of carcinoma *in situ* not even when the growth extended down into the glands In 11 cases histolog ical examination of serial section showed some down growth of intraepithelial cancer in the cervical glands In this group also roentgen examination showed no evidence of the lesion.

The appearance of the cervix 6 months to 1 year after the operation is shown in Table III The cervix healed with complete restoration of the original form and of the mucosal relief No widening capable of causing cervical insufficiency was observed. The women with the widest internal os after operation (10 mm on 12th day of cycle) later gav birth to a healthy boy weighing 2660 g Neither pregnancy nor delivery was complicated.

The return of the radiological appearances to normal was seen very soon after the operation When the patients were examined as early as 3–4 weeks after the operation the appearances were the same except that there were occasionally pockets in the cervical wall which were no long r demonstrable at later ex

Table III. *Radiological Appearance of Cervix 6 Months to 1 Year after Conization. (Italics give the number of cases where cancer in situ could not be proven histologically.)*

Day of Cycle	Width of Cervix: Small	Average	Large	Internal Os (mm)	Mucosal Relief: No Visible Plicae	Slight Plication	Well Defined Plicae	Abnormal Findings
0–12		28		2 10 (mean 4.6)	12+3	8+2	3	0
>12		14		3–8 (mean 5.3)	6 3	2+1	0+*1*	0

Postoperative functional state of cervix

The operation did not appear to interfere with later function of the cervix. Thus, of 32 of the women in whom pregnancy might be expected, 13 (41 per cent) became pregnant. Of the remaining patients, 3 had radiologically diagnosed bilateral hydrosalpinges before the operation, 5 used contraceptive pills after the operation, 1 had a hysterectomy because of a recurrence of carcinoma in situ and 3 were more than 43 years old. These 13 women in whom pregnancy was possible had 17 pregnancies within the first 4 years after the operation. Two of the pregnancies ended in spontaneous abortion. None of the children were born before term and none of the pregnancies were complicated.

The functional state of the cervix after conization has received little consideration. Eliasson *et al.* (1964) reported that abortion occured in 26 per cent of 31 pregnancies in women who had been subjected to conization. Of 23 pregnancies in 56 women who were below 35 years and who had previously undergone conization because of cancer *in situ* Boyd *et al.* (1963) found that 22 per cent terminated in abortion. Green (1966) reported 30 pregnancies among 231 women who had undergone conization because of cancer *in situ* and concluded that the operation did not impair fertility as 43 per cent of those women in whom conception could be expected became pregnant. In his series the abortion frequency was 18.4 per cent. He thought that abortion, premature delivery and cervical dystocia were more common after conization.



2a 2b

Fig 2. A 16 year old 0-para with cancer *in situ* Normal pregnancy 4 years after operation Cervix before (a) and 6 weeks after (b) conization. Normal findings

aminations These pockets had presumably been caused by in advertent cuts in cervical muscle by the tip of the surgeon s knife That healing was rapid and good is illustrated in Figs 1–3 A cervical pocket" is shown in Fig 4

Histological findings

All of the cones removed at repeat operation showed regenera tion of the lining epithelium and of the crypts and glandular ducts In four cases well developed plicae palmatae were seen in transverse sections of the cervix In one case no plicae could be seen with certainty and in the remaining 2 cases the sections would not allow evaluation in this respect.

For help with the hist logical exam nations we thank As P of Berge Dpt of Pathol (Head. Prof F Linell) Malmö



Fig 4 A 27 year old Para II Cervix after operation. A cervical pocket, 10 mm by 4 mm, with its base downward (↓)

SUMMARY

Forty four women aged 16–49 years were operated upon in 1962–1963 with cold-knife conization without subsequent suturing, because of suspected cancer of the uterine cervix stage 0 The patients were subjected to hysterosalpingography before, and at 1 and at 6 to 12 months after the operation.

The cervix soon recovered its preoperative roentgen appearances There was no widening of the cervical canal Formation f new glands was shown histologically

By 1967 13 of the 32 women who were capable of conceiving had a total of 17 pregnancies (conception rate 41 per cent) Only 2 of these pregnancies ended in spontaneous abortion. All the pregnancies were normal and there were no premature births.


3a


3b


3c

Fig. 3. A 22 year old 0-para with cancer in situ. Cervix before (a), 6 weeks after (b) and 6 months after (c) conization. Normal findings.



Fig. 4. A 27 year old Par II. Cervix after operation. A cervical pocket, 10 mm by 4 mm, with its base downward. (↓)

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a



b



c

Fig 3 A 22 year old 0-para with cancer *in situ*. Cervix before (a), 6 weeks after (b) and 6 months after (c) conization. Normal findings.

Ulstein, Magnar Knut, Acta obst. et gynec. scandinav. 48, 267, 1969
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TORSION OF THE PREGNANT HUMAN UTERUS

BY

MAGNAR KNUT ULSTEIN

Torsion of the pregnant human uterus is a very rare condition. Only 129 cases have been described. Towards the end of pregnancy a slight rotation of the uterus may occur normally. In 80 per cent of cases this physiological rotation occurs in a clockwise direction, and more than 30 degrees is considered pathological (*Nowosielskie* and *Henderson* 1960). Further rotation will give rise to symptoms depending on the degree of torsion, the stage of pregnancy, and the speed at which the torsion itself develops. Two cases have been described with torsion of 540 degrees (*Olow* 1910 and *Schindler* 1919) and one case with as much as 720 degrees (*Syme* 1906).

Case report

The patient was 31 years old, admitted Feb. 11, 1965. Forceps delivery in 1959. The infant was deformed and died after 4 days. Two years later she had a normal delivery. The third pregnancy, the one under consideration, was normal until last weeks before term. She then developed mild toxaemia. Transverse lie had been present for several weeks. Correction to head presentation was easily done, but as soon as the patient lay on her back the foetus slipped back to transverse lie. She had no abdominal pains or other striking symptoms. Vaginal examination showed a closed cervix, an unusual finding in a gravida III at term. It was not possible to reach the membranes or the presenting part when the foetus was pressed down into the pelvic inlet. The foetus seemed to be full term. Radiography showed transverse lie and normal pelvis. Three days after the expected date of delivery she was operated on. On opening the abdomen the uterus presented at once a rather unusual picture. The peritoneum over the lower uterine segment was wrinkled. The fimbrial end of one tube was visible deep on the left side. There were some tense strands over the lower part of the cervix. The round ligaments

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the 9th month of pregnancy In 2 cases of uterine myoma the stage of pregnancy was not mentioned. In cases with no pathological findings there was an increasing incidence towards term with 1 case before the 5th month of pregnancy 3 in the 5th–6th months, 7 in the 7th–8th months and 11 in the 9th month of pregnancy When abnormal foetal presentation was found, torsion occurred in the last 2 months of pregnancy In cases of uterine anomalies and of adhesions, torsion was most common in the 2nd third of pregnancy

Symptoms

The most common symptom was abdominal pain, found in 107 cases Only 6 patients were symptom-free. When the patient had labour pains, partus prolongatus was an important finding (23 cases) Symptoms of shock were found in 31 patients vaginal bleeding in 10 patients, urinary tract symptoms in 18 and intestinal symptoms in 34 patients. In most cases the symptoms developed acutely indicating that torsion occurred as a sudden catastrophe Exceptions to this were the 6 symptom free cases in which Caesarean section was carried out for other reasons. In 28 cases torsion developed in connection with partus. Sudden body movements of the mother have been suggested as predisposing factors (*Björkenheim*, 1965)

Diagnosis

No special symptoms or signs can lead to diagnosis. Torsion was usually a surprising finding at operation in cases presenting urgent reasons for laparotomy without an exact diagnosis having been made In 7 cases diagnosis was not made until autopsy On clinical examination the cervix is closed. The presenting part of the foetus cannot be reached. The round ligament may be palpated as a tender strand on the anterior surface of the uterus. Differential diagnosis early in pregnancy can include all acute abdominal conditions. Later in pregnancy the most important differential diagnosis are rupture of the uterus ablatio placentae or acute hydramnion.

were not visible. The uterus was rotated 180 degrees, and the right adnexa were pulled across to the left side. The left tube and ovary were concealed by the uterus on the right side. The uterus was easily manipulated to normal position Nothing pathological was found in regard to the uterus or adnexa. Caesarean section was carried out in the usual way with a low transverse incision and a living male child, weighing 3870 g, was extracted breech first. There were no postoperative complications.

Torsion of the nonpregnant uterus has been described by *Times* in 1861 and later by *Virchow* in 1863 Both instances were noted in combination with uterine fibroids at autopsy In 1876 *Labbé* published the first case of torsion of the pregnant human uterus. More recently there have been several reports of individual cases and a few surveys The total number of described cases is now 130 including the present one Seventeen cases in the literature were insufficiently detailed to be considered in the following brief survey

Aetiology

In 1904 *Barrows* maintained "No tumour no torsion. *Robinson* and *Duvall* (1931) modified this to No uterine abnormality no torsion *Nesbitt* and *Corner* (1956) state "No pelvic pathology torsion unlikely However it appears that torsion can occur without extant pathological changes in the uterus adnexa or pelvis.

Uterine myoma were present in 36 cases uterine anomalies in 16 adhesions in 14 abnormal presentation of the foetus in 8 pelvic deformity in 3 scarred cervix in 2 placenta praevia in 3 ovarian tumour in 7 and interstitial pregnancy in 2 cases. However the group with no pathological changes was also large with 22 cases in all In the 8 cases with abnormal foetal presentation the uterus adnexa and pelvis were normal

The parity was unknown in 4 cases 46 were primigravida and 63 were plurigravida The rotation was clockwise in 66 cases anti-clockwise in 39 and unknown in 8 cases. The torsion was most common in the cervical region

In cases of uterine myoma torsion occurred most frequently early in pregnancy 19 cases before the 5th month of pregnancy 6 cases in the 5th–6th months 3 in the 7th–8th months and 7 in

the 9th month of pregnancy In 2 cases of uterine myoma the stage of pregnancy was not mentioned. In cases with no pathological findings there was an increasing incidence towards term with 1 case before the 5th month of pregnancy 3 in the 5th–6th months 7 in the 7th–8th months and 11 in the 9th month of pregnancy When abnormal foetal presentation was found, torsion occurred in the last 2 months of pregnancy In cases of uterine anomalies and of adhesions torsion was most common in the 2nd third of pregnancy

Symptoms

The most common symptom was abdominal pain, found in 107 cases. Only 6 patients were symptom-free. When the patient had labour pains, partus prolongatus was an important finding (23 cases) Symptoms of shock were found in 31 patients, vaginal bleeding in 10 patients, urinary tract symptoms in 18 and intestinal symptoms in 34 patients. In most cases the symptoms developed acutely indicating that torsion occurred as a sudden catastrophe Exceptions to this were the 6 symptom free cases in which Caesarean section was carried out for other reasons. In 28 cases torsion developed in connection with partus. Sudden body movements of the mother have been suggested as predisposing factors (*Björkenheim*, 1965)

Diagnosis

No special symptoms or signs can lead to diagnosis. Torsion was usually a surprising finding at operation in cases presenting urgent reasons for laparotomy without an exact diagnosis having been made In 7 cases diagnosis was not made until autopsy On clinical examination the cervix is closed. The presenting part of the foetus cannot be reached. The round ligament may be palpated as a tender strand on the anterior surface of the uterus. Differential diagnosis early in pregnancy can include all acute abdominal conditions. Later in pregnancy the most important differential diagnosis are rupture of the uterus, ablatio placentae, or acute hydramnion.

Treatment

In the majority of cases treatment has been laparotomy Nine patients were not operated on In 47 cases Caesarean section was carried out, in 26 hysterectomy in 13 myomectomy in 6 ovariec tomy and in 12 cases laparotomy and detorsion of the uterus. The treatment to be recommended is laparotomy in time Early in pregnancy detorsion and if possible removal of the cause should be carried out. Where the foetus is viable Caesarean section should be performed.

Prognosis

Both foetal and maternal mortality have been high The respective figures being 43 per cent and 15 per cent. When the torsion occurred after the 28th week of pregnancy the foetal mortality was 37 per cent. In 15 cases there was both foetal and maternal death. The maternal mortality was highest when torsion occurred in the later stages of pregnancy *Nesbitt* and *Corner* (1956) point out that both foetal and maternal mortality were highest in the cases first published The same authors showed that maternal mortality increases with increasing degree of torsion Caesarean section was performed in 34 of the cases with torsion of the uterus in the last month of pregnancy Of these 3 mothers and 4 infants died. The 5 untreated cases in the same month of pregnancy all died both mothers and infants

Discussion

Whether transverse lie predisposes to torsion or vice versa is con jectural. It may be pointed out that transverse lie does lead to asymmetry of the uterus Irregularity in uterine form seems to be an important aetiological factor (*Imrie* 1966 *Mitchell* and *Gar rett*, 1960) In our case transverse lie had been present for several weeks before admission. It can be noted that all of the published symptom free cases were at term. The diagnosis was made by chance at Caesarean section carried out for other reasons. In 2 cases there was transverse lie in 2 adhesions in 1 pelvic deformity and in 1 case uterine myoma

Although the condition is very uncommon, one should be aware of it, for if the uterus is opened before detorsion is carried out, massive bleeding may occur

SUMMARY

A case is reported with 180 degrees of torsion of the uterus in a full term pregnancy There were no symptoms. The diagnosis was made by chance at Caesarean section carried out because of transverse lie

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THE EFFECT OF HISTAMINASE INHIBITION ON THE CONCENTRATION AND DISTRIBUTION OF ^{14}C HISTAMINE IN BLOOD DURING PREGNANCY

BY

ÅKE TÖRNQVIST

The histaminolytic activity of blood plasma is considerably increased during human pregnancy (*Marcou et al* 1938 *Ahlmark* 1944) *Lindberg* (1963) showed that the concentration of ^{14}C histamine in arterial blood of pregnant women was approximately 50 per cent lower than that of non pregnant women during intravenous infusions of equal doses of isotope labelled histamine Using the same technique *Lindberg* and *Törnqvist* (1966) studied the blood concentration of histamine in pregnant women after inhibition of the enzyme histaminase by means of intramuscular injection of aminoguanidine. The pattern of the histamine metabolites *i e* the absence of imidazoleacetic acid in the blood, indicated that the inhibitory effect of the aminoguanidine was complete *Lindberg* and *Tornqvist* (1966) also found that that the arterial blood concentration of ^{14}C histamine in pregnant women was higher after histaminase inhibition as compared with the conditions when the enzyme was left intact (*Lindberg*, 1963)

In view of these results it was surprising that previous investigators (*Janowitz* and *Grossman* 1949 *Wicksell* 1949 *Kullander* 1952 *Clark* and *Tankel* 1954) did not find any difference in the biological effects of exogenous histamine between pregnant and non pregnant women

To elucidate this problem further the effects of infused histamine on the heart rate and on the forearm blood flow were studied in pregnant and non pregnant women before and after inhibition of histaminase (*Törnqvist* 1968 a and b) In these

investigations Törnqvist found significantly higher effects of the infused histamine on the heart rate and on the forearm blood flow in the non-pregnant than in the pregnant women. The responses to histamine were also significantly increased in the pregnant group after the inhibition of histaminase.

The aim of the present investigation was to study the concentration of ^{14}C histamine in arterial blood after infusion of isotope labelled histamine under similar experimental conditions to those which were used in the studies of the biological effects of exogenous histamine (*Törnqvist* 1968 a and b). In investigations by *Lindell* and *Wiske* (1961) and by *Lindell* and *Westling* (1962) most of the ^{14}C histamine recovered from the blood, after intravenous infusion of isotope labelled histamine, was found in the plasma. In the present investigation the concentration of ^{14}C histamine therefore has been studied in the plasma and the blood cell fraction as well as in total blood. The disappearance of ^{14}C histamine in total blood *in vitro* was also investigated.

Materials

Fifteen physically healthy pregnant women were studied. They were admitted to the clinic for legal abortion and sterilization and were in the 17th to the 22nd week of pregnancy counting from the first day of the last menstrual period. Information regarding these pregnant women is given in Table I.

The infused isotope labelled histamine was ^{14}C histamine (histamine dihydrochloride labelled in the 2 position of the imidazole ring) obtained from the Radiochemical Centre Amersham, England. The non-isotopic carrier was histamine dihydrochloride obtained from E. Merck A. G. and the aminoguanidine used for inhibition of the enzyme histaminase was aminoguanidine sulphate (Eastman Organical Chemicals).

Methods

In an attempt to avoid discomfortable histamine effects all subjects received 50 mg promethazine (Lergigan, Recip) intramuscularly half an hour before the beginning of the investiga-

Table I. *Description of Cases and Infusion Rates of ^{14}C Histamine*

Case	Age in Years	Duration of Pregnancy in Weeks	Body Weight Kg	Infusion Rate of ^{14}C Histamine in cpm	Infusion Rate of ^{14}C Histamine in cpm kg of Body Weight
1	35	19	69	39 000	565
2	38	20	60	35,000	580
3	31	18	86	41,000	480
4	34	18	91	23 000	250
5	40	22	56	23,000	410
6	40	19	68	45,000	660
7	34	17	59	44,000	750
8	38	17	69	42,000	610
9	31	19	67	22,000	330
10	41	18	71	39 000	550
11	29	20	71	41,000	80
12	38	17	61	–	–
13	37	19	78	–	–
14	34	19	67	–	–
15	42	20	39	–	–

Infusion rate of histamine in counts per minute (cpm)

tion. All investigations were done in the morning with the subjects fasting

Infusion of histamine Isotope labelled histamine was infused intravenously into the right arm by means of a motor-driven syringe The histamine was dissolved in 0.9 per cent saline. Infusion rates of 12 and 21 μg of histamine base per minute were used. Infusion periods of 6 minutes were chosen to correspond with the experimental conditions in previous investigations on some biological effects of exogenous histamine (*Törnqvist* 1968 a and b) All subjects recieved two infusions of histamine with 30 minutes interval In the histamine free intervals physiological saline solution was infused, at a low speed to prevent clotting in the needle and the vein.

Inhibition of histaminase The histaminase was inhibited by intramuscular injection of 0.2 mg of aminoguanidine sulphate per kg of body weight immediately after the first infusion of histamine was finished. The injected solution contained 10 mg of

aminoguanidine sulphate per ml of sterilized water. The histaminase level in plasma was studied in 6 pregnant women before and after the injection of aminoguanidine. A biological testing method described by *Ahlmark* (1944) showed that the plasma histaminase activity half an hour after the injection of the aminoguanidine in all cases was decreased almost to a non-pregnant value. This and other investigations (*Brody*, *Lindberg* and *Törnqvist*, unpublished) showed that 30 minutes were sufficient for an almost complete inhibition of the histaminase activity in human plasma.

Sampling of blood. Immediately before stopping the infusion of histamine blood was withdrawn from a polythene catheter which had been inserted previously in the left brachial artery under local anaesthesia as described by *Berneus*, *Carlsten*, *Holmgren* and *Seldinger* (1954). Ten ml samples were usually taken for analyses. Blood samples were always obtained with heparine treated syringes. To separate the blood cells from the plasma the blood was centrifugated for 8 minutes at a rate of 6000 rev/minute at room temperature (the diameter of the centrifuge head was about 24 cm).

Assay of histamine. The plasma and the blood cell fraction were transferred 8 minutes after sampling to previously weighed glass tubes with known volumes of non-isotopic carrier. The distribution of the infused histamine was studied in 11 cases. In 6 of these the concentration of isotope labelled histamine in total blood was also estimated. In these 6 cases one part of the blood was mixed with carrier immediately after sampling. Another part of the same blood sample was mixed with carrier 8 minutes after sampling. In this way it was possible to study the histaminolysis *in vitro* at room temperature during the time necessary to separate the plasma and the blood cell fraction. The concentration of histamine was measured by Schayer's isotope dilution technique (*Schayer* and *Cooper*, 1956, *Lindell* and *Schayer*, 1958). The blood samples were mixed with carrier equivalent to 206 mg of the corresponding picrate. Trichloracetic acid was added to give a final concentration of about 5 per cent, thus precipitating the proteins. The samples were filtered and the acid removed from the filtrate with ether. The filtrate was saturated with

anhydrous Na_2SO_4 and made strongly alkaline with NaOH The histamine was extracted with n butanol and the butanol fraction was extracted with HCl. The HCl extract was evaporated to dryness. The histamine-dihydrochloride so obtained was dissolved in water and picric acid. The histamine-dipricate was converted to histamine-dihydrochloride by passage through a Dowex anion exchange column and was made to react with p-iodophenyl sulphonyl chloride (pipsylchloride) Pipsylhistamine was recrystallized and treated with activated charcoal several times until three successive crystallizations showed fairly constant radioactivity The radioactivity was measured at infinite thickness in a flow counter operated with methane. At least 1000 counts were taken after every recrystallization. The constant value for each sample was corrected for background radioactivity and expressed as counts per minute per ml of blood (cpm/ml) One μg of the ^{14}C histamine base used in this study gave about 2000 cpm above background.

Results

In 11 cases (cases 1–11) the concentration of ^{14}C histamine was estimated in the plasma and in the blood cell fraction before and after inhibition of histaminase The results are given in Table II. The concentration of isotope labelled histamine in cpm/ml increased in both fractions after inhibition of histaminase

In 6 cases (cases 6–11) the investigation was extended to estimate the concentration of isotope labelled histamine in total blood These results are also given in Table II In most cases the concentration of histamine in total blood was increased after inhibition of the histaminase This increase however was much smaller in those blood samples which were immediately mixed with non isotopic carrier (total blood I) than in the samples which were mixed with carrier 8 minutes after sampling (total blood II) Before inhibition of histaminase there was a considerable disappearance of histamine in the blood samples which were mixed with carrier 8 minutes after sampling After inhibition of histaminase however there was no corresponding histaminolysis. The influence of this histaminolysis *in vitro* on the

Table II Concentration of 14C Histamine in Plasma Blood Cell Fraction nd Total Blood in Counts per Minute per ml (cpm/ml) Duri g I ravenous I fusion of Labelled Histamine

Case	Infusion Rate of Histamine		Before Inhibition of Histaminase				After Inhibition of Histaminase			
	Micro-gram/min	pm	Plasma	Blood Cell Fraction	Total Blood I	Total Blood II	Plasma	Blood Cell Fraction	Total Blood I	Total Blood II
1	21	39,000	4 7	2.4			13 0	4 4	–	
2	21	35,000	7 4	2 5			11.5	5.1		
3	21	41,000	5.9	2.5			13.9	6 0		–
4	12	23,000	3 9	1.9			8.7	3.4		–
5	12	23,000	2.4	1 4			8 9	3 7		–
6	21	45,000	2 1	1 7	7.0	1 1(2 0)	15.8	6.8	11 7	11.9(11.9)
7	21	44,000	7 0	4	9 2	4 4(5.4)	17.5	7 1	9 1	12.9(13.5)
8	21	42,000	1 7	1 5	5.9	1.5(1 6)	14.6	6.4	11.0	10 4(11.0)
9	12	22,000	2 6	1 4	4 0	1 7(2.1)	6.2	3.1	4.9	4 8(4.9)
10	21	9,000	7 1	3 0	8.7	13._(5.3)	18.7	12.5	11.6	12.7(16 2)
11	21	41,000	6 5	3.3	6 0	4 9(5 3)	15 4	5.6	6.3	12.3(11.5)

Total blood I represents the blood fraction which was immediat ly mixed with non isotopi carrier and total blood II th fraction which was mixed with carrier 8 minutes after sampling

The figures in brackets indicat the histamine concentration in total blood which was calculated from the corresponding values in plasma and blood cell fraction

anhydrous Na_2SO_4 and made strongly alkaline with NaOH. The histamine was extracted with n butanol and the butanol fraction was extracted with HCl. The HCl extract was evaporated to dryness. The histamine-dihydrochloride so obtained was dissolved in water and picric acid. The histamine-dipricate was converted to histamine-dihydrochloride by passage through a Dowex anion exchange column and was made to react with p-iodophenyl sulphonyl chloride (pipsylchloride). Pipsylhistamine was recrystallized and treated with activated charcoal several times until three successive crystallizations showed fairly constant radioactivity. The radioactivity was measured at infinite thickness in a flow counter operated with methane. At least 1000 counts were taken after every recrystallization. The constant value for each sample was corrected for background radioactivity and expressed as counts per minute per ml of blood (cpm/ml). One μg of the ^{14}C histamine base used in this study gave about 2000 cpm above background.

Results

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In 6 cases (cases 6–11) the investigation was extended to estimate the concentration of isotope labelled histamine in total blood. These results are also given in Table II. In most cases the concentration of histamine in total blood was increased after inhibition of the histaminase. This increase however was much smaller in those blood samples which were immediately mixed with non isotopic carrier (total blood I) than in the samples which were mixed with carrier 8 minutes after sampling (total blood II). Before inhibition of histaminase there was a considerable disappearance of histamine in the blood samples which were mixed with carrier 8 minutes after sampling. After inhibition of histaminase however there was no corresponding histaminolysis. The influence of this histaminolysis *in vitro* on the

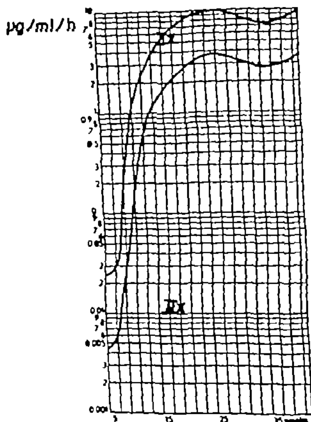


Fig. 1 Histaminolytic activity in blood plasma of pregnant woman befor (Ix) and after (IIx) inhibition of histaminase by means of intramuscular injection of aminoguanidine

Abscissa weeks of pregnancy calculated from the first day of the last menstrual period

Ordinat histaminolytic activity expressed in g of inactivated histamine per ml of plasma and per hour

plasma was measured, before and after inhibition of histaminase *in vivo* using a biological technique described by *Ahlmark* (1944)

The results were all similar and showed a marked reduction of the histaminolytic activity after the injection of aminoguanidine. Fig. 1 shows one of these studies.

Table III *Distribution of ^{14}C Histamine Between Plasma and Blood Cell Fraction in Percentage of Histamine Recovered During Infusion of Labelled Histamine*

Case	Before Inhibition of Histaminase		After Inhibition of Histaminase	
	In Plasma	In Blood Cell Fraction	In Plasma	In Blood Cell Fraction
1	76	24	82	18
2	80	20	74	26
3	72	28	72	28
4	65	35	76	24
5	70	30	78	22
6	62	38	75	25
7	72	28	79	21
8	57	43	74	26
9	71	29	71	29
10	76	24	68	32
11	76	24	81	19

concentrations of histamine in plasma and blood cell fraction in the present investigation will be discussed later

It is possible to calculate the concentration of histamine in that total blood fraction which was mixed with carrier 8 minutes after sampling from the ratio

$$\frac{\text{cpm (plasma)} + \text{cpm (blood cell fraction)}}{\text{ml (plasma)} + \text{ml (blood cell fraction)}}$$

These values are given within brackets in Table II In most cases the calculated and the directly measured concentrations of histamine correspond well In case 10 however the directly measured value is much higher than the calculated one. In this case there was good reason to believe that this single sample was contaminated with radioactive material in the laboratory

The distribution of the recovered isotope labelled histamine between plasma and blood cell fraction is given in Table III About 1/3–1/5 of the recovered histamine was found in the blood cell fraction both before and after inhibition of histaminase

In 6 cases (cases 10–15) the histaminolytic activity of the

composition continues outside the organism and that histaminolysis *in vitro* is much greater in the blood of pregnant than in that of non-pregnant women. *Lindberg* (1963) also showed that this type of histaminolysis can be almost completely inhibited if aminoguanidine is added to the blood immediately after sampling. A detailed analysis of the quantitative importance of histaminolysis *in vitro* in measuring plasma histamine concentrations has not been made in the present investigation. Such an analysis is taking place where aminoguanidine is added to the blood immediately after sampling and before the separation of plasma and blood cells. It has not yet been finished as very few patients have been available.

Histaminolysis *in vitro* in total blood was studied in those cases where part of the blood was mixed with carrier immediately after sampling and part was left in the syringe at room temperature for 8 minutes before carrier was added. Before the injection of aminoguanidine the histaminolysis during this time was considerable but it was almost abolished after the inhibition of histaminase. The degree of *in vitro* histaminolysis also showed considerable variation among the cases in the present investigation.

In an investigation by *Lindell* and *Västo* (1961) it was shown that the concentration of histamine during the infusion of histamine to dogs, rose rapidly in plasma but more slowly in the blood cell fraction. When the infusion of histamine to these dogs was stopped, the histamine concentration fell more rapidly in the plasma than in the blood cell fraction. The fact that histaminolysis is not uniform in plasma and blood cells increases the difficulty of evaluating the concentrations of histamine in the present investigation.

A comparison of the histamine concentrations, before and after inhibition of histaminase in the total blood samples which were immediately analysed is less subject to error since the effects of the histaminolysis *in vitro* are probably small. In most cases there was an increase in the total blood concentrations of histamine after the enzyme inhibition. That the concentration in at least one of the cases was not increased at all, may possibly be due to a compensatory increase in the methylation of histamine (*Lindberg* and *Törnqvist* 1966).

Discussion

The infusion of histamine has well known pharmacological effects in man especially on the circulatory system. Earlier investigators in this field were unable to show any measurable increase in histamine concentration in total blood (*Rose* 1940) and in plasma (*Emmelin et al* 1941 *Adam et al* 1950 and 1954) during intravenous infusion, even if the infused histamine produced marked biological effects. In investigations in cat (*Emmelin et al* 1941) and in man (*Adam* 1954) biological indicators were considered to be more sensitive than current methods for histamine estimation in blood for detecting an increase in the concentration of free histamine in total blood and plasma. With more refined methods for estimation of the histamine content in total blood and plasma however *Adam et al* (1957) demonstrated that the histamine concentration in plasma was increased during infusion of histamine. With the aid of *Schayer's* isotope dilution technique labelled histamine could be measured accurately (*Lindell* and *Schayer* 1958) and it was possible to correlate the biological effects with the actual concentration of histamine in the blood during histamine infusion (*Lindell* and *Westling*, 1962) *Lindell* and *Westling* (1962) showed that increase in the pulse rate and flushing of the face were found even if the increase in concentration of histamine in the plasma was very small.

The aim of the present investigation was to study the concentration of ^{14}C histamine in total blood plasma and blood cell fraction during an infusion of isotope labelled histamine under similar experimental conditions to those which were used in earlier investigations on some circulatory effects of exogenous histamine before and after inhibition of histaminase (*Tornqvist* 1968 a and b)

In the present investigation there was a considerable increase in the concentration of histamine in both plasma and blood cell fraction after inhibition of histaminase The lower concentrations of histamine before administration of aminoguanidine may be due either to an increased destruction of histamine *in vitro* during the time necessary for the separation of the plasma and the blood cell fraction or to an increased destruction of histamine in the circulating blood. *Lindberg* (1963) showed that histamine de-

before and after inhibition of histaminase. In all these cases the histaminolytic activity was decreased to a non-pregnant level after the enzyme inhibition.

In all blood samples analysed for ^{14}C histamine there was a general tendency towards increased concentration of histamine after inhibition of histaminase.

Before inhibition of histaminase there was a considerable disappearance of histamine from those total blood samples which were analysed 8 minutes after sampling. No corresponding histaminolysis occurred after inhibition of histaminase.

The distribution of the recovered histamine was studied and both before and after inhibition of histaminase about 1/3–1/5 of the histamine was found in the blood cell fraction.

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The distribution of infused isotope labelled histamine between the plasma and the blood cell fraction has been studied previously in non pregnant dogs by *Lindell* and *Viske* (1961) and in man by *Lindell* and *Westling* (1962) In these investigations only 1/3–1/4 of the recovered histamine was found in the blood cells. The correspondance between these investigations and the present investigation is fairly good even if the increased histaminolytic activity in the pregnant women might have modified the distribution of infused histamine in the present study

Judging by the study of the histaminolytic activity of the plasma before and after injection of aminoguanidine the method for inhibition of histaminase in the present investigation may be efficient. The histaminolytic activity was measured by the conventional method using bioassay of the remaining histamine on guinea pig ileum after incubation of plasma with non-isotopic histamine. In all the 6 pregnant women studied there was a marked reduction of histaminolytic activity after injection of aminoguanidine.

The results of the present investigation show a general tendency towards increasing concentrations of histamine in total blood plasma and blood cell fraction after inhibition of histaminase *in vivo* with otherwise identical experimental conditions. This supports the earlier observations of an increased response to infused histamine of pregnant women after inhibition of histaminase (*Törnqvist* 1968 a and b)

SUMMARY

Eleven pregnant women received infusions of ^{14}C histamine before and after inhibition of histaminase by means of an intra muscular injection of aminoguanidine In all cases arterial blood was withdrawn during the infusion and after centrifugation, the concentration of isotope labelled histamine in the plasma and the blood cell fraction was estimated In 6 of the cases arterial blood samples withdrawn simultaneously were analysed and the concentration of histamine in total blood was estimated both immediately and after 8 minutes at room temperature

In 6 cases the histaminolytic activity of plasma was estimated

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FOREARM BLOOD FLOW DURING NORMAL PREGNANCY STUDIED BY VENOUS OCCLUSION PLETHYSMOGRAPHY AND ^{133}XENON MUSCLE CLEARANCE

BY

SVEN SPETZ AND INGE JANSSON

Marked changes in the distribution of blood flow to the skin and skeletal muscles have been shown to occur during normal preg nancy (*Abramson Flachs* and *Fierst* 1943 *Herbert Banner* and *Wakim* 1954 1958 *Burt* 1950 *Spetz* 1964 *Ginsburg* and *Duncan* 1967)

Venous occlusion plethysmography is a convenient method for studying alterations in peripheral blood flow This method was applied in all the above mentioned studies of skin and skeletal muscle blood flow during normal pregnancy

Changes in total forearm blood flow measured by venous oc clusion plethysmography reflect to a large extent alterations in muscular blood flow (*Grant* and *Pearson* 1938 *Allen Barcroft* and *Edholm*, 1946) Changes in skin blood flow however may also be of importance when recording variations of total forearm blood flow

A method which made it possible to differentiate between the blood flow in different tissue components of the same organ was introduced by *Kety* in 1949 when he demonstrated that the rate of disappearance of the locally injected radioactive isotope sodium–24 was proportional to the local blood flow in the tissues. The chemically inert radioactive gases krypton–85 and xenon 133 later have proven to be even more useful in this respect (*Lassen* 1964 *Holzman Wagner Ilo Rabinowitz* and *Zierler* 1964)

The present investigation was initially undertaken in an at

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the repeated plethysmographic examinations could influence the following ^{133}Xe clearance determination, the order was reversed and ^{133}Xe clearance measured first. Furthermore the water temperature of the plethysmograph was reduced to 26 C to minimize the influence of skin blood flow on total forearm blood flow. This part of the study was used when calculating the correlation between the plethysmographic and the ^{133}Xe clearance values. The water temperature used in each case is stated in Table II.

Plethysmography

Venous occlusion plethysmography was applied to the right forearm in the same manner as described in detail by Spetz (1964). During measurements the proximal cuff pressure was maintained at least 20 mm Hg below the diastolic arterial pressure as determined in the left arm by the conventional sphygmomanometer and auscultation of Korotkow's sound. In most experiments the proximal cuff pressure was 40 mm Hg. The distal cuff pressure was kept at about 60 mm Hg to prevent venous backflow from the hand and not above the arterial systolic pressure as usually recommended, because prolonged supra-arterial pressure may cause considerable pain and discomfort to the test subject. Several experiments revealed that the values for forearm blood flow were not influenced by this change in technique. At least ten consecutive measurements were performed on each test subject with intervals of about one minute. The mean value was calculated and taken as total forearm blood flow at rest, expressed in ml per 100 ml of forearm tissue per minute.

Xe clearance method

^{133}Xe dissolved in isotonic saline was obtained at first from the Radiochemical Centre, Amersham, England, later from AB Atomenergi, Studsvik, Nyköping, Sweden. 0.05–0.10 ml of the solution with an activity of about 30–50 microcurie was injected slowly (injection time 10–15 seconds) intramuscularly into the extensor portion of the forearm musculature. A needle no. 20 (outer diameter 0.5 mm) was used and inserted, at an oblique angle to the skin surface, to a depth of about 1.5 cm. The needle

tempt to evaluate the validity of the ^{133}Xe clearance method in comparison with venous occlusion plethysmography One of us (*Spetz* 1964) has demonstrated a marked increase in total forearm blood flow during the course of normal pregnancy Thus another purpose of this study was to elucidate in which tissue component of the forearm these changes occur in the skeletal muscles in the skin or both.

Material

Forty-one women who were between the 16th and 43rd weeks of pregnancy were studied. Their mean age was 25 years with a range from 16 to 44 years. The cases of early pregnancy were women about to undergo legal abortion on socio-medical grounds. Those in later pregnancy were women under observation because of some minor complication of pregnancy not affecting the circulatory system. ^{133}Xe clearance in the extensor muscles of the forearm was studied in 38 subjects. In 26 of these cases forearm blood flow was studied simultaneously by venous occlusion plethysmography In the remaining three cases only plethysmography was performed as the ^{133}Xe clearance examinations had to be discarded because of technical error Thirteen non-pregnant subjects were also studied, both methods being used in 11 of these cases. This group consisted mainly of women treated at the hospital because of salpingitis which had subsided completely at the time of examination. Their mean age was 24 years with a range from 18 to 42 years

Methods

The observations were not started until the test subjects had been resting in bed for at least 30 minutes The room temperature was maintained at 22 C During the earlier part of the investigation the plethysmographic examination was performed first and a water temperature of 34 C chosen as this seems to be the optimal temperature (*Barcroft* and *Edholm* 1945) About ten minutes after the arm was taken out of the plethysmograph the ^{133}Xe clearance measurement was started. As we felt that

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where λ is the muscle/blood partition coefficient of xenon. This has been calculated to be about 0 7 for skeletal muscle of the dog (Conn 1961) If this figure is taken to be valid also for human muscle tissue the original equation can be written

$$MBF = -70 \; \frac{dQ/dt}{Q} \text{ or}$$

$$MBF = -70 \; \ln 10 \; \frac{d \log Q}{dt}$$

where, as a logarithmic potentiometer is used, $-d \log Q/dt$ can be expressed as the fraction of one decade which the tangent of the elimination curve decreases in one minute (D) The ultimate equation for calculating the muscle blood flow then becomes

MBF = 70 2.3 D = 161 D ml per 100 g muscle per minute (*Lassen et al.* 1964)

If the basic assumption of complete diffusion equilibrium of xenon between tissue and blood is fulfilled and the tissue area under investigation is evenly perfused, the recorded ^{133}Xe clearance curve can be expected to be mono-exponential, i.e. a straight line with logarithmic recording, and D easily calculated. This was, however very seldom the case, but in most instances the first 5–6 minutes of the recording was straight or could be approximated to a straight line (Fig. 1) *Kjellmer Lindbjerg, Pierovsky* and *Tonnesen* (1967) calculated muscle blood flow from this initial slope in experiments on the isolated gastrocnemius muscle of cats and found it to correlate well with the directly measured blood flow *Zierler* (1965) proposed a method of calculating blood flow from the area under the curve independent of its shape *Kjellmer et al.* however showed that this method gave about 15 per cent lower values than the initial slope method, which in turn yielded muscle blood flow values only 62 per cent of the directly measured blood flow when intramuscular injection of the isotope was used. By a different labelling technique completely avoiding any tissue damage *Tonnesen* and *Sejrsen* recently demonstrated that the initial part of the clearance rate from a local depot of xenon is practically identical to the blood flow measured directly

was not withdrawn until 30 seconds after the injection to avoid possible backflow along the needle track. The elimination rate of the injected ^{133}Xe was followed by an external scintillation detector with a NaI (Tl) crystal. A cylindrical lead collimator was used with an inner diameter of 5 cm and a length of about 9 cm from the crystal to the aperture which was placed immediately above the injection site. The scintillation detector was coupled to a spectrometer set to record the peak intensity of the gammaradiation from ^{133}Xe (81 KeV). A time constant of 3 seconds was chosen. The gammaspectrometer was connected to a logarithmic potentiometer writing on linear paper. The initial counting rate was usually between 10^4 and $3 \cdot 10^4$ counts per minute. The clearance rate was continuously recorded during 6–12 minutes.

Calculations

If an inert and freely diffusible substance such as xenon is introduced into a tissue it will establish equilibrium with the blood stream through the tissue very rapidly and according to the Fick principle the rate by which it is removed from the tissue will be proportional to the rate of blood flow provided the lymphatic outflow can be neglected (*Kety* 1960). This can be expressed with the equation

$$dQ/dt = F(C_a - C_v)$$

where Q is the quantity of xenon in the region at any given time (t), F the rate of blood flow and C_a and C_v the concentrations of xenon in arterial and venous blood. Since the xenon is diluted by venous return from other tissues and removed almost completely from the blood via the expired air, C_a is negligible and the equation can be reduced to

$$dQ/dt = -F \cdot C_v$$

F can be substituted for $\frac{MBF \cdot W}{100}$ where MBF is the muscle blood flow in the small area investigated per 100 g of tissue and W the tissue weight in grams. C_v can be substituted for $\frac{Q}{W} \cdot \frac{1}{\lambda}$

where λ is the muscle/blood partition coefficient of xenon. This has been calculated to be about 0 7 for *skeletal muscle of the dog* (*Conn* 1961) If this figure is taken to be valid also for human muscle tissue the original equation can be written

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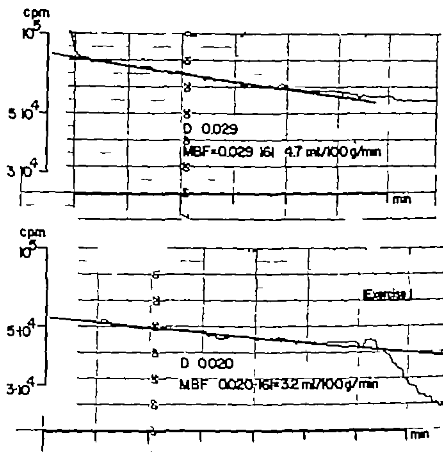


Fig. 1 Original charts of two recordings of ^{133}Xe clearance from forearm muscle with lines drawn in to illustrate the straight part of the curve At the end of the lower recording the test subject produced hyperaemia by one minute s muscular exercise

(*Tonnesen* 1968) Thus MBF was calculated from the equation given above When drawing the line of slope care was taken to include as much as possible of the initial part of the curve Curves with a very marked multi-exponential course were dis carded because it was impossible to make a straight line fit the curve No attempt was made to divide the curves into two or more components as described by e g *Haggendal Nilsson and Norbäck* (1965) for brain tissue or *Lundgren* (1967) for small intestine as the meaning of this in the case of skeletal muscle is obscure

Table I. *Forearm Blood Flow Measured by Plethysmography (Water Temperature 26°C) and* ^{133}*Xe Clearance in Forearm Muscle in Non-Pregnant Subjects*

Subj. No.	Blood Flow Plethysm. ml/100 ml/min ± SE	^{133}Xe Clearance Slope/min (D)	Flow ml/100 g/min
57	3.9 ± 0.22	0.028	4.5
58	1.8 ± 0.08	0.018	2.9
59	2.2 ± 0.16	0.014	2.3
60	3.9 ± 0.27	0.023	3.8
62	3.7 ± 0.08	0.029	4.7
66	1.4 ± 0.04	0.018	2.6
69	1.2 ± 0.06	0.017	2.7
70	3.3 ± 0.18	0.019	3.4
78	1.4 ± 0.02	0.018	2.8
79	2.8 ± 0.10	0.023	3.6
83	2.0 ± 0.06	0.016	2.6
90		0.020	3.2
92		0.015	2.4
Mean ± SE	2.5 ± 0.32		3.2 ± 0.22

Statistical methods

The mean value standard error of the mean (SE) correlation coefficient and regression line were calculated by conventional methods (*Brownlee* 1965) In the text and tables the mean value is gi en ± SE. The significance of differences was evaluated by the t test. When the variances were unequal the Welch procedure was applied (*Brownlee* 1965) When not otherwise stated the tests were performed at the 5 per cent level of significance

Results

The results in the non-pregnant subjects are given in Table I. The mean forearm blood flow recorded with the plethysmograph was 2.5 ± 0.32 ml/100 ml/min and the mean ^{133}Xe clearance in forearm muscle 3.2 ± 0.22 ml/100 g/min. Comparison by a

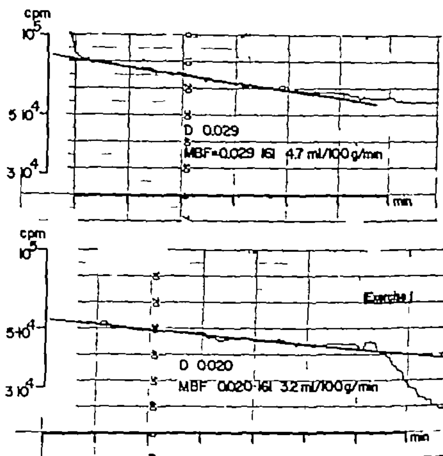


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Table III. Total Forearm Blood Flow and ^{133}X Clearance in Forearm Muscle in Non-Pregnant Subjects and at Different Periods of Pregnancy Mean Values ± Standard Error of Mean

Method	Non-Pregnant Subjects	16–24 Weeks of Pregnancy	25–36 Weeks of Pregnancy	37–43 Weeks of Pregnancy
Plethysm.	2.3 ± 0.32	3.5 ± 0.66	5.6 ± 0.42	7.7 ± 0.76
ml/100 ml/min	n = 11	n = 9	n = 7	n = 13
^{133}Xe clearance	3.2 ± 0.21	4.3 ± 0.54	3.1 ± 0.64	3.3 ± 0.22
ml/100 g/min	n = 13	n = 13	n = 5	= 20

t test on differences between measurements in the same case shows that the difference is statistically significant.

The results in the pregnant subjects are collected in Table II. The mean total forearm blood flow of all pregnant women regardless of gestation period was 5.9 ± 0.52 ml/100 ml/min. This value is significantly higher than that found in the non pregnant subjects ($P < 0.001$). The mean ^{133}Xe clearance rate in forearm muscle in the pregnant women was 3.6 ± 0.24 ml/100 g/min. This is not significantly different from the mean ^{133}Xe clearance rate in the non-pregnant group.

In Table III the mean values for resting forearm blood flow and for ^{133}Xe muscle clearance at different periods of pregnancy are given. The mean forearm blood flow recorded by plethysmograph between the 16th and 24th weeks of pregnancy was not significantly different from that of non-pregnant subjects. The mean values for total forearm blood flow between the 25th and 36th, and the 37th and 43rd weeks of pregnancy were both significantly higher than the mean value of the non-pregnant group ($P < 0.001$). There was, however, no significant difference between the mean values of muscle blood flow determined by ^{133}Xe clearance at different periods of gestation when compared with the non-pregnant group.

In Fig. 2 the mean values are plotted against the duration of pregnancy. It is evident from the figure that the forearm blood flow recorded with the plethysmograph and the ^{133}Xe clearance in forearm muscle were about equal until about the 24th week

Table II. *Forearm Blood Flow Measured by Plethysmography and ^{133}Xe Clearance in Forearm Muscle in Healthy Pregnant Women*

Subj No	Week of Pregnancy	Water Temp Pleth C	Blood Flow Plethysm. ml/100 ml/min ± SE	^{133}Xe Clearance	
				Slope/min (D)	Flow ml/100 g/min
9	16			0.026	4.3
25	16	34	2.5 ± 0.05	0.040	6.4
6	17			0.034	5.4
5	18			0.016	2.6
65	18	26	7.6 ± 0.24	0.051	8.3
89	18			0.013	2.1
84	19	26	4.1 ± 0.17	0.034	5.5
32	20	34	3.1 ± 0.12	0.019	3.1
73	20	26	2.6 ± 0.20	0.019	3.1
63	21	26	4.0 ± 0.12	0.028	4.4
72	21	26	1.3 ± 0.05	0.009	1.5
61	22	26	1.4 ± 0.03	0.019	3.1
76	22	26	5.1 ± 0.29	0.036	5.7
68	25	26	6.2 ± 0.28		
34	26	34	5.4 ± 0.17	0.030	4.8
80	30	26	5.2 ± 0.37	0.012	1.9
85	30	26	3.6 ± 0.19	0.011	1.8
53	31	34	5.0 ± 0.28		
46	32	34	6.8 ± 0.18	0.017	2.7
77	35	26	6.7 ± 0.59	0.029	4.7
45	37	34	9.9 ± 0.31	0.029	4.7
49	37	4	6.1 ± 0.16	0.010	1.6
87	37			0.020	3.2
21	38			0.015	2.4
71	38	26	2.4 ± 0.16	0.017	2.7
81	38	26	5.4 ± 0.18	0.020	3.2
8	39			0.023	3.7
54	39	34	10.6 ± 0.40	0.020	3.2
67	39	26	9.3 ± 0.21	0.027	4.3
74	39	26	9.4 ± 0.27	0.022	3.5
82	39	26	10.7 ± 0.40	0.017	2.7
86	39	26	4.4 ± 0.21	0.025	4.0
88	39			0.014	2.2
31	40			0.020	3.2
43	40	34	10.3 ± 0.57	0.030	4.8
56	40	26	5.0 ± 0.21	0.034	5.5
75	40	26	8.5 ± 0.53	0.018	2.9
91	40			0.013	2.1
52	41	34	6.0 ± 0.22		
3	43			0.025	4.0
19	43			0.018	2.9
Mean ± SE			5.9 ± 0.52		3.6 ± 0.24

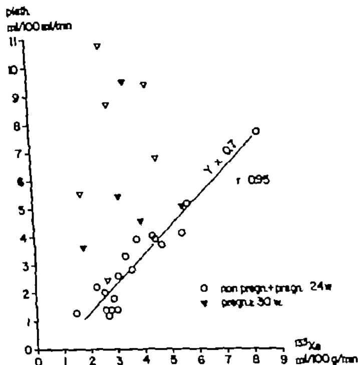


Fig. 3. Relation between total forearm blood flow determined by plethysmography and ^{133}Xe clearance in forearm muscle in pregnant and non-pregnant subjects.

and pregnant cases up to 22 weeks of gestation, the other of cases 30 weeks pregnant or more (there were no cases between 23 and 29 weeks in this series) an interesting pattern appears. For the first group there is a linear correlation between the plethysmographic forearm blood flow and the ^{133}Xe muscle clearance the correlation coefficient (r) being 0.95 ($P < 0.001$) The calculated regression line is $Y = 1.0x - 0.7$ meaning that the muscle blood flow calculated from the Xe clearance rate was on average 0.7 ml/min. higher than the total forearm blood flow determined by plethysmography but that an increase of one unit in blood flow with one method meant an increase of one

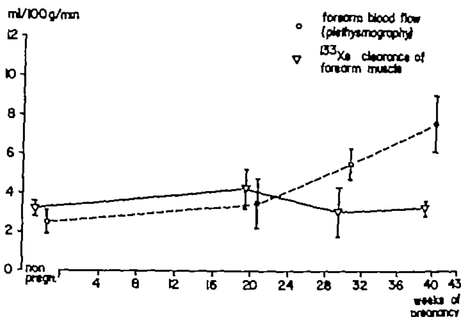


Fig 2. Forearm blood flow determined by plethysmography and ^{133}Xe clearance in forearm muscle in non-pregnant subjects and at different stages of pregnancy The mean values are given ±2 SE.

of pregnancy From then on the total forearm blood flow showed a progressive rise while the ^{133}Xe clearance remained fairly constant throughout the pregnancy

Correlation study

In this part of the study were included all pregnant and non pregnant cases where the water temperature of the plethysmograph was maintained at 26 C and the plethysmographic as well as the Xe clearance recordings were considered accurate There were 29 such pairs of observations In Fig. 3 the relationship between blood flow values determined by the two methods is illustrated. There seemed at first to be a fairly uniform distribution of the plotted values in the coordinate system and no definite correlation could be established. If however the series is divided into two groups one composed of all non pregnant cases

ml/min. This difference may perhaps be explained by technical differences in performing the forearm blood flow measurements.

In non-pregnant subjects total forearm blood flow is mainly muscle blood flow as the muscles occupy about 60 per cent of the forearm tissue volume and the skin and subcutaneous tissue only about 10 per cent (*Abramson* and *Ferris* 1940 *Cooper Edholm* and *Mottram* 1955) The rest of the forearm volume is bones and tendons, the blood flow in which is low and constant and can be neglected. Changes in hand blood flow however reflect to a large extent changes in skin blood flow as 30 per cent of the hand volume is skin and only 16 per cent muscle (*Abramson* and *Ferris* 1940) There is general agreement (*Burt* 1950 *Spetz* 1965 *Ginsburg* and *Duncan* 1967) that there is an increase in skin blood flow in late pregnancy of about 15 ml/100 ml/min as judged from hand plethysmography

Thus skin blood flow must make a contribution to the rise in total forearm blood flow in late pregnancy but it seemed to us improbable that it could be responsible for the whole increase. Kety's (1949) tissue clearance method to determine local blood flow seemed suitable to evaluate the part played by the muscle mass in this increase Kety originally used "Na but the radioactive inert gas "Xe later has been shown to give more reliable information (*Lassen* 1964) as it is freely diffusible through the cell membrane and can be assumed to establish equilibrium between tissue and blood ery rapidly *Lassen Lindbjerg* and *Munck* (1964) and *Lassen Lindbjerg* and *Dahn* (1965) applied the "Xe clearance method to the skeletal muscles of the leg in cases of occlusi e vascular disease and found the method to permit a clearcut differentiation between normal and diseased legs Recently *Siggaard Andersen* and *Bonde Petersen* (1967) and *Tonnesen* (1968) have demonstrated a good correlation be tween 'Xe muscle clearance and plethysmography of the calf under conditions of submaximal asodilatation.

When the "Xe clearance method was applied to the forearm muscles during pregnancy no change in the calculated muscle blood flow could be demonstrated, despite the rise in total forearm blood flow during the second half of pregnancy However it was possible to demonstrate a strong linear correlation (r =

unit also with the other After the 30th week of pregnancy the total forearm blood flow did not show any correlation with ¹³³Xe muscle clearance.

Discussion

Changes in peripheral circulation during pregnancy as reflected by total forearm blood flow have been studied by a number of investigators *Abramson et al* (1943) and *Ginsburg* and *Duncan* (1967) were not able to demonstrate any change in resting forearm blood flow during pregnancy The latter authors however found a progressive increase in hand blood flow towards the end of pregnancy *Burt* (1950) made this latter observation but she also noted an increase in forearm blood flow as did *Herbert et al.* (1954 1958)

One of us (*Spetz* 1964) has studied forearm blood flow at different stages of normal pregnancy under strictly standardized conditions by means of venous occlusion plethysmography A progressive increase in forearm blood flow was shown to occur following an exponential function from midpregnancy until term The mean forearm blood flow in early pregnancy was 2 8 ml/100 ml/min as compared with 12.3 ml at term, meaning a four to fivefold increase

The present study has confirmed the finding by *Spetz* (1964) The rise in forearm blood flow during the second half of pregnancy however was not of the same magnitude This may be explained by differences in water temperature of the plethysmograph. Spetz used a temperature of 36 C while in the present study lower temperature levels 34 C and 26 C were chosen. At 34 C the resting mean forearm blood flow at term was 9 0±0 9 ml/100 ml/min (n = 5) and at 26 C 6 9±1 0 ml/100 ml/min (n = 8) The difference suggests that the lower water temperature by reducing skin blood flow brought about a decrease in total forearm blood flow Nevertheless there was a more than threefold increase in peripheral blood flow at the end of pregnancy This result is in contrast with the findings of *Ginsburg* and *Duncan* (1967) who reported a remarkably low forearm blood flow throughout the pregnancy *1.9-2.7 ml/100*

must be assumed to take place through open capillaries not involved in blood-tissue exchange. As xenon is freely diffusible it should be independent of a pressure gradient across the capillary wall. Thus, if a non-nutritional capillary blood flow exists, ^{133}Xe clearance should measure this too. There is however the possibility of unequal perfusion of different parts of the same muscle, in which case ^{133}Xe clearance would not reflect the conditions in the entire muscle.

A comment of the second alternative requires some mathematical considerations. As skin constitutes only 30 per cent of the hand volume an increase of 15 ml/100 ml/min in hand blood flow must mean a rise in skin blood flow of about 50 ml per min and 100 ml skin, neglecting the blood flows of the other tissues of the hand. If this figure is applied to the skin of the forearm, which occupies about 10 per cent of the forearm volume, it can account for a rise in total forearm blood flow of about 5 ml/100 ml/min. This is very close to the actually observed increase in the present study

Thus, the results of the present study are in favour of an increased skin blood flow being the cause of the observed rise in forearm blood flow during late pregnancy This conclusion, however is based on the assumption that the change in forearm skin blood flow is equal to that of the hand. This may not be true In any case a general increase in skin blood flow of that magnitude would mean that the skin of the body would take an unreasonably great share of the cardiac output at the end of pregnancy

SUMMARY

1 Resting forearm blood flow determined by venous occlusion plethysmography and ^{133}Xe clearance in the extensor musculature of the forearm was studied in forty-one pregnant and thirteen non-pregnant healthy subjects.

2 The mean forearm blood flow recorded with the plethysmograph in the non-pregnant group was 2.5 ml, in midpregnancy 3.5 ml and in late pregnancy 7.7 ml/100 ml/min. The corresponding mean ^{133}Xe clearance values of forearm muscle was 3.2, 4.3

0.95) between forearm blood flow recorded by the plethysmograph and ^{133}Xe clearance of forearm muscles in non pregnant and early pregnant subjects. This indicates that ^{133}Xe clearance reflects individual variations in forearm muscle blood flow under these circumstances.

To be able to discuss these contradictory results it is necessary to review the current conception of skeletal muscle blood flow (*cf Folkow* 1967). From a functional point of view each vascular circuit in the body consists of various series-coupled sections the local and central nervous regulation of which may vary considerably 1) "Windkessel" vessels (large and middle sized arteries) 2) resistance vessels with a precapillary and a post capillary section, 3) exchange vessels, *i e* true capillaries with their precapillary sphincter vessels and ultimately 4) capacitance vessels (venules and veins). In addition shunt vessels have been demonstrated in some tissues *e g* in skin. The tone of the resistance vessels determines the peripheral resistance to blood flow and hence the magnitude of regional blood flow. This is measured by venous occlusion plethysmography. The tone and relation between pre and postcapillary resistance vessels determine to a great extent mean hydrostatic capillary pressure and the rate of transcapillary filtration. Changes in tone of the precapillary sphincter vessels regulate the number of capillaries open for blood flow and capillary exchange. As the locally injected tracer has to be eliminated through perfused capillaries ^{133}Xe clearance must be assumed to measure the rate of blood flow in these capillaries. This capillary blood flow is mainly regulated by the local metabolic demands of the tissues.

Thus in the non pregnant and early pregnant state this capillary blood flow is equal to the total regional blood flow. The results in late pregnancy suggests that the total regional blood flow then greatly exceeds the capillary blood flow of the muscles. This may have two possible explanations. The first is that blood may pass through vessels not involved in muscle nutrition. The second is that the rise in skin blood flow is greater than supposed.

As there are no true arterio-venous anastomoses in muscle tissue (*Walder* 1968) any shunting of blood that may occur

must be assumed to take place through open capillaries not involved in blood-tissue exchange. As xenon is freely diffusible it should be independent of a pressure gradient across the capillary wall. Thus if a non-nutritional capillary blood flow exists, ^{133}Xe clearance should measure this too. There is, however the possibility of unequal perfusion of different parts of the same muscle, in which case ^{133}Xe clearance would not reflect the conditions in the entire muscle.

A comment of the second alternative requires some mathematical considerations. As skin constitutes only 30 per cent of the hand volume an increase of *15 ml/100 ml/min in hand blood* flow must mean a rise in skin blood flow of about 50 ml per min and 100 ml skin, neglecting the blood flows of the other tissues of the hand. If this figure is applied to the skin of the forearm, which occupies about 10 per cent of the forearm volume, it can *account for a rise in total forearm blood flow of about 5 ml/100* ml/min. This is very close to the actually observed increase in the present study

Thus, the results of the present study are in favour of an increased skin blood flow being the cause of the observed rise in forearm blood flow during late pregnancy This conclusion, however is based on the assumption that the change in forearm skin blood flow is equal to that of the hand. This may not be true In any case a general increase in skin blood flow of that magnitude would mean that the skin of the body would take an unreasonably great share of the cardiac output at the end of pregnancy

SUMMARY

1 Resting forearm blood flow determined by venous occlusion plethysmography and ^{133}Xe clearance in the extensor musculature of the forearm was studied in forty-one pregnant and thirteen non-pregnant healthy subjects.

2 The mean forearm blood flow recorded with the plethysmograph in the non pregnant group was 2.5 ml, in midpregnancy 3 5 ml and in late pregnancy 7 7 ml/100 ml/min. The corresponding mean ^{133}Xe clearance values of forearm muscle was 3.2, 4.3

and 3.3 ml/100 g/min. The total regional blood flow of the forearm as determined by plethysmography showed a progressive rise from midpregnancy until term, while "Xe clearance in forearm muscle was unchanged throughout the pregnancy and equal to that of non pregnant subjects

3 There was a strong positive correlation (r = 0 95) between forearm blood flow determined by plethysmography and "Xe muscle clearance when the groups of non pregnant subjects and pregnant women up to 22 weeks of gestation were taken together In late pregnancy no relationship between blood flow values determined by the two methods could be demonstrated. The reason for this is discussed.

Acknowledgements

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^{133}XENON CLEARANCE IN THE MYOMETRIUM OF PREGNANT AND NON PREGNANT WOMEN

BY

INGE JANSSON

The complicated vascular anatomy of the uterus makes direct measurements of uterine blood flow during human pregnancy extremely difficult. The electromagnetic flowmeter used by *Assali Rauramo* and *Peltonen* (1960) measures the blood flow only in one uterine artery and its application implies considerable risks and uncertainties. The total uterine blood flow per unit weight of tissue has been calculated from the Fick principle using nitrous oxide (*Assali Douglass Baird Nicholson* and *Suyemoto* 1953 *Metcalfe Romney Ramsey Reid* and *Burwell* 1955) or 4-aminoantipyrine (*Huckabee* 1962) as test substances This technique however is based on repeated sampling of blood from a representative uterine vein, involving hazardous catheterization procedures or venous cannulation at caesarean section.

Kety's (1949) method of measuring regional blood flow from the disappearance rate of a locally injected radioactive tracer (^{24}Na) made a less traumatic approach possible This method has been applied to the pregnant uterus by several investigators (*Browne* and *Veall* 1953 *Morris Osborn* and *Payling Wright* 1955 *Johnson* and *Clayton* 1957 *Moore* and *Myerscough* 1957 *Dixon Browne* and *Davey* 1963) all demonstrating a reduced ^{24}Na clearance in the myometrium in preeclampsia in comparison with normal pregnancy Their results have been criticized for a number of reasons (*Huckabee* 1962 *Hytten* and *Leitch* 1964 *Assali* and *Morris* 1964) One objection is that the tracer is de-

posited in a very small area assuming homogeneity of the tissue, and another one that the clearance rate is proportional not only to the blood flow but also to the diffusion rate of the tracer across the capillary membrane. While some of the criticism is applicable also to ^{133}Xenon, a radioactive gas which in recent years has replaced ^{24}Na in measurements of local tissue blood flow (*Lassen Lindbjerg* and *Munck* 1964) this tracer has one definite advantage over ^{24}Na. It is chemically inert and freely diffusible through the cell membranes allowing calculation of tissue clearance in absolute figures of local blood flow. Furthermore, in spite of local injection of a small depot of the tracer the ^{133}Xe clearance rate has been shown to reflect regional blood flow in skeletal muscle relatively well (*Kjellmer et al* 1967 *Tonnesen* 1968 *Spetz* and *Jansson* 1969)

^{133}Xe has been used to measure myometrial blood flow in late pregnancy by *Munck Lysgaard Pontonnier Lefèvre* and *Lassen* (1964) *Lysgaard and Lefèvre* (1965) and by *Guilhem Pontonnier* and *Pontonnier* (1965) As had earlier been the experience with ^{24}Na, they found a wide scatter of the recorded clearance values. In a methodological study on cases of legal abortion *Falk Forkman* and *Lindell* (1967) could demonstrate that this scatter was largely dependent of the distance of the injection site from the placenta.

The present study was undertaken in order to reveal possible variations of ^{133}Xe clearance in the myometrium at different periods of pregnancy in each case determining the site of the placenta. As a comparison myometrial ^{133}Xe clearance in the non-pregnant state and in the puerperium was studied.

Material

Forty-six measurements of myometrial ^{133}Xe clearance were performed in 40 women between the 10th and 43rd weeks of pregnancy. Their ages varied from 19 to 43 years. Twenty measurements in the *first half of pregnancy* were carried out in 17 women admitted to the clinic for legal abortion on psychiatric or sociomedical grounds. In 13 of these cases sterilization was also performed and the placental site could be determined at

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cases where a clearcut differentiation of injection site could be obtained between placental and non-placental myometrium (myometrium proper) were included in the study

This method of transperitoneal injection through the exposed peritoneum was also used in three puerperal cases, where sterili zation was performed.

In the non-pregnant cases the uterus had to be exposed during the injection but was well covered by adjacent structures in the pelvis during the measurement.

2. *Cases with placental localisation by arteriography* The test subject was resting in bed for at least 30 minutes before the measurement was started. As the placental site was known, a suitable place for the injection of xenon could be chosen, either placental myometrium or myometrium proper The injection was performed transabdominally with the same thin needle as de scribed above and without anaesthesia, as the penetration of the needle caused very little or no discomfort. When the uterine wall was reached the needle point was inserted about 5–10 mm further into the myometrium. The injection technique was the same as described above and the elimination rate of the injected ^{133}Xe recorded in the same way An identical procedure was used in those cases where the placental site was not known and in 7 of the puerperal cases.

The adiation dose for each measurement is extremely low *Lassen* (1965) calculated that 100 Ci ^{133}Xe exposed the foetus and ovaries to a dose of 0 4 millirad at a distance of 10 cm.

Calculations Assuming a tissue/blood partition coefficient of xenon for myometrium of 0 7 which is stated to be an approximately correct figure for most tissues except fat (*Andersen* and *Ladefoged* 1967) the local myometrial blood flow (MBF) can be calculated from the equation given by *Lassen et al* (1964)

MBF = 161 D ml per 100 g of tissue per minute.

D i the fraction of one decade by which the logarithmic elimina tion curve decreases in one minute With an even perfusion of the tissue the elimination curve should form a straight line This was almost always the case in measurements on non-pregnant and puerperal uteri but in experiments on pregnant uteri the urve ften showed a multi-exponential course. Most often, how

hysterotomy Twenty six measurements were undertaken in 23 women who were 25–43 weeks pregnant. In 12 of these cases the placenta was located by pelvic arteriography in 5 cases at elective caesarean section. The indication for placentography was slight to moderate ante partum bleeding with suspicion of placenta praevia. Complete placenta praevia was diagnosed in one case and a minor degree in three others. The bleeding had subsided completely in all cases at the time of the ^{133}Xe clearance examination.

Eleven non pregnant women, aged 17–40 years, were investigated during abdominal operations for ovarian cysts or sterilization. In all these cases the uterus looked normal. Ten women, aged 22–41 years were studied in the immediate post partum period.

Methods

1 *Laparotomy cases* (legal abortions with sterilization, caesarean sections non pregnant cases) Intubation anaesthesia with controlled breathing through a non-rebreathing system was used in order to make recirculation of xenon negligible A low midline incision was made down to the peritoneum, which was not incised. About 50 μCi ^{133}Xe dissolved in 0 10–0 15 ml isotonic saline was injected through the intact peritoneum into the adjacent uterine wall. The needle 5 cm long with an outer diameter of 0.5 mm, was introduced at an oblique angle to the uterine wall to a depth of about one cm. No aspiration was made and care was taken to inject the solution slowly (injection time at least 10 seconds) and not to withdraw the needle until 15–30 seconds after the injection was ended. The gamma-radiation from the injected isotope was recorded by the same set of scintillation detector gammaspectrometer and logarithmic potentiometer writing on linear paper described in an earlier paper (*Spetz and Jansson* 1969) The aperture of the lead collimator covered with sterile draping, was placed immediately above the injection site. When the measurement was completed the abdominal cavity was entered and the relation between the injection site and the placenta was determined at the subsequent hysterotomy Only those

Table I. *Reproducibility of 133X. Clearance Method*

Case		133Xe Clearance ml/100 g/min		
		1st Determ.	2nd Determ.	Difference
N.F	non-pregnant	25.8	28.6	−2.8
K.A.	non-pregnant	17.3	15.9	+1.4
L.O	non-pregnant	15.7	14.2	+1.5
E.A.	non-pregnant	9.0	8.2	+0.8
B.N	non-pregnant	40.5	39.7	+0.8
A.O	non-pregnant	26.8	20.0	+6.8
M.N	pregnant	20.2	24.6	−4.4
R.J	puerperal	43.0	38.6	+4.4

involved in such a procedure in this type of tissue. For a more detailed discussion of the calculation of blood flow from the slope of the elimination curve *cf.* *Spetz* and *Jansson* (1969)

Statistical methods. Conventional statistical methods were used for calculation of mean value and standard error of the mean (SE). In the text and tables the mean values are given ± SE. The significance of differences between groups was tested by one way analysis of variance and Scheffe's method for multiple comparisons. Bartlett's test was used to test the equality of variances. For details see *Brownlee* (1965). When not otherwise stated the tests were performed at the 5 per cent level of significance.

The reproducibility of the 133Xe clearance method was evaluated by a test on squared differences between paired observations (Table I). The difference between two observations in the same case is not statistically different from zero.

Results

Non pregnant uteri

The mean of 18 measurements of myometrial 133Xe clearance in 11 women was 23.0 ± 2.98 ml/100 g/min (Table II). The range was as great as 8.2–40.5 ml/100 g/min. There was no difference in clearance rate between different parts of the uterine body as can be seen from Table II and Fig. 2. The reason for the great variation could not be demonstrated in this small series. It is, however, interesting to note that the mean myometrial 133Xe

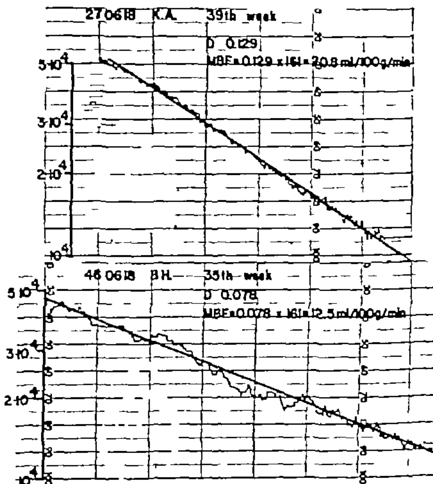


Fig 1 Myometrial ^{133}Xe clearance in two pregnant women. In both cases the placenta was located by pelvic arteriography In the upper recording the placenta was situated in the anterior wall where the xenon injection was made. In the lower recording the placenta was situated in the posterior wall and the xenon injection made in the anterior wall *i.e* in myometrium proper The ordinate denotes the externally recorded gamma-radiation expressed in counts per minute (cpm) The distance between each heavy vertical line is one minute.

ever the initial part of the curve was satisfactorily straight until 70–80 per cent of the injected ^{133}Xe was eliminated. The myometrial blood flow was then calculated from this initial slope (Fig 1) If it was impossible to make a straight line fit the curve the experiment was discarded. No att mpt was made to divide the curves into different components because of the uncertainty

Table II. ^{133}Xe Clearance in Myometrium in Non-Pregnant Women

Patient Name	Age Years	No. of Pregnancies	Day of Menstr Cycle	^{133}Xe Clearance Slope/min (D)	Flow ml/100 g/min	Mean	Site of Injection
N P	40	3	27	0.160	25.8		fundus
				0.184	28.6	28.6	body
				0.196	31.5		isthmus
K A	33	1	23	0.107	17.3	16.6	body
				0.099	15.9		isthmus
L O	23	0	10	0.098	15.7	15.0	body
				0.068	14.2		isthmus
E A	17	0	22	0.056	9.0	8.6	body
				0.051	8.2		isthmus
B N	24	2	pre-menstr.	0.253	40.5	40.1	body
				0.247	39.7		isthmus
A O	18	0	5	0.167	26.8	23.4	fundus
				0.124	20.0		body
F A	30	4	14	0.200	32.2		body
S O	30	5	prolif phase	0.153	24.8		body
K A	24	0	18?	0.082	13.2		body
M A	30	4	19	0.210	33.8		body
M B	23	2	7	0.104	16.8		body
				Mean ± SE	23.0 ± 2.98		

Isthmus here denotes the lower segment of the uterine body at the level of the vesico-uterine peritoneal reflection.

Pregnant uteri

The results in the pregnant group are seen in Tables III, IV and V. The mean ^{133}Xe myometrial clearance in 40 pregnant women regardless of the placental site was 18.6 ± 1.37 ml/100 g/min. Again there was a wide range from 4.5 to 39.5 ml. If however the isotope was injected in the myometrium at the placental implantation site the mean clearance rate was 25.8 ± 1.67 ml/100 g/min (range 19.3–39.5) and if it was made in non-placental myometrium (myometrium proper) the mean clearance rate was 10.6 ± 0.97 ml/100 g/min (range 4.5–16.1).

It is obvious from table V that there was no difference in

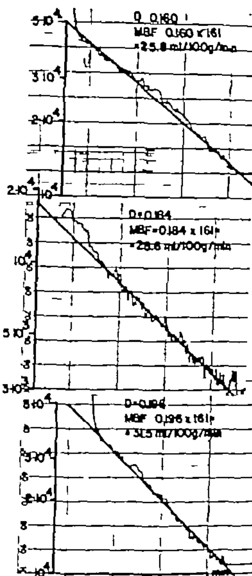


Fig. 2. Three recordings of myometrial ^{133}Xe clearance in the same non-pregnant uterus. The figure illustrates the reproducibility of the clearance method and the fact that there is no essential difference in clearance rate between different parts of the non-pregnant uterus. The ordinates denote gamma-radiation in cpm.

clearance in 7 parous women was 27 6 ml/100 g/min as compared with 15 1 ml in 4 non parous women. The lowest recorded value 8 2 ml/100 g/min, was found in a 17 years old gravida 0 with a small juvenile uterus.

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Patient Name	Age Years	No. of Pregnancies	Day of Menstr Cycle	^{133}Xe Clearance Slope/min (D)	Flow ml/100 g/min	Mean	Site of Injection
N P	40	3	27	0.160	25.8		fundus
				0.184	28 6	28.6	body
				0.198	31.5		isthmus
K.A.	33	1	23	0.107	17.3	16 6	body
				0.098	15.9		isthmus
L O	23	0	10	0.098	15.7	15.0	body
				0.088	14.2		isthmus
E A.	17	0	22	0 056	9.0	8.6	body
				0.051	8.2		isthmus
B N	24	2	pre-menstr	0.253	40.5	40.1	body
				0.247	39.7		isthmus
A O	18	0	5	0 167	26.8	23.4	fundus
				0.124	20.0		body
E A	30	4	14	0 200	32.2		body
S O	30	5	prolif phase	0.153	24.8		body
K.A.	24	0	18?	0.082	13.2		body
M.A.	30	4	19	0.210	33.8		body
M B	23	2	7	0 104	16.8		body
				Mean ± SE	23 0 ± 2.98		

Isthmus here denotes the lower segment of the uterine body at the level of the vesico-uterine peritoneal reflection.

Pregnant uteri

The results in the pregnant group are seen in Tables III IV and V The mean ^{133}Xe myometrial clearance in 40 pregnant women regardless of the placental site was 18 6 ± 1.37 ml/100 g/min. Again there was a wide range from 4.5 to 39.5 ml. If however the isotope was injected in the myometrium at the placental implantation site the mean clearance rate was 25 8 ± 1 67 ml/100 g/min (range 19.3–39.5) and if it was made in non-placental myometrium (myometrium proper) the mean clearance rate was 10.6 ± 0.97 ml/100 g/min (range 4.5–16.1)

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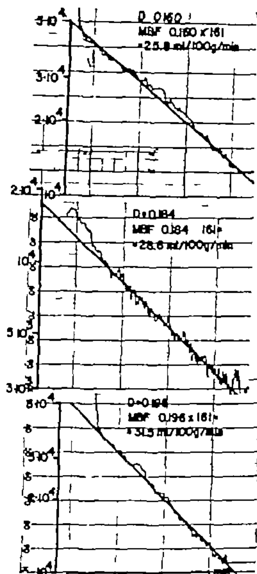


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clearance in 7 parous women was 27.6 ml/100 g/min as compared with 15.1 ml in 4 non parous women. The lowest recorded value 8.2 ml/100 g/min, was found in a 17 years old gravida 0 with a small juvenile uterus.

Table IV ^{133}Xe Clearance in Myometrium in Late Pregnancy

Patient Name	Age Years	Preg-nancy No.	Week of Preg-nancy	^{133}Xe Clearance Slope/Min (D)	^{133}Xe Clearance Flow ml/100 g/min	Site of Injection
E.A.	21	1	25	0.129	19.5	plac. myometr
L.N.	19	1	26	0.083	13.4	not determined
I.I.	35	4	29	0.120	19.3	plac. myometr
			38	0.157	25.2	plac. myometr
L.A.	26	9	32	0.059	9.5	myometr proper
			35	0.098	15.8	myometr proper
B.H.	21	2	35	0.078	12.5	myometr proper
B.O.	24	1	37	0.059	8.7	myometr proper
K.E.	23	2	37	0.091	14.6	myometr proper
A.W.	24	1	37	0.243	39.0	plac. myometr
I.B.	31	3	37	0.140	22.6	plac. myometr.
G.E.	27	2	38	0.089	14.3	not determined
K.A.	17	1	38	0.132	21.2	plac. myometr
I.H.	23	3	38	0.080	12.9	not determined
B.H.	20	1	39	0.090	14.5	myometr proper
K.A.	40	3	39	0.129	20.7	plac. myometr
R.R.	27	3	39	0.042	6.8	myometr proper
P.H.	25	3	39	0.051	8.2	myometr proper
M.B.	26	1	39	0.245	39.5	plac. myometr
M.L.	23	3	39	0.084	13.4	not determined
L.D.	25	1	40	0.100	16.1	myometr proper
I.K.	19	1	40	0.160	25.8	plac.myometr
M.J.	19	1	41	0.066	10.6	myometr proper
A.S.	21	2	41	0.160	25.8	not determined
E.L.L.	19	1	43	0.055	8.8	not determined
				0.061	11.3	not determined
				Mean ± SE	17.6 ± 1.82	

g/min, i.e. to the level of myometrium proper. This coincided with a steep fall in the urinary oestriol excretion.

Puerperal uteri

The mean of 11 measurements of myometrial ^{133}Xe clearance in the first few days post partum was 32.1 ± 3.99 ml/100 g/min (Table VI). There were great individual variations in this group

Table III. ^{133}Xe *Clearance in Myometrium in Early and Midpregnancy*

ient me	Age Years	Preg nancy No.	Week of Preg nancy	^{133}Xe Clearance			Site of Injection
				Slope/min (D)	Flow ml/100 g/min	Mean	
	39	4	10	0.028	4.5		myometr proper
.	40	5	11	0.143	23.0		plac. myometr
I	40	2	14	0.126	20.2	22.4	plac. myometr
				0.153	24.6		plac. myometr
	34	3	14	0.082	13.2		myometr proper
	31	5	14	0.218	35.0		plac. myometr
	37	3	14	0.150	24.2		plac. myometr
:	35	4	15	0.091	14.6		plac. site not
				0.114	18.3	19.2	determined
				0.154	24.8		
.	38	4	16	0.191	30.8		plac. myometr
L.	38	3	16	0.150	24.2		not determined
.	26	6	17	0.163	26.2		not determined
.	38	5	19	0.123	19.9		plac. myometr
	40	5	19	0.041	6.6		myometr proper
.	24	2	20	0.147	23.6		not determined
.	19	1	21	0.042	6.8		myometr proper
	36	10	22	0.163	26.3		plac. myometr
.	38	7	22	0.127	20.4		plac. myometr
.	38	4	23	0.077	12.4		myometr proper
				Mean ± SE	19.9 ± 2.08		

myometrial ^{133}Xe clearance between early and midpregnancy on one hand and late pregnancy on the other either with respect to placental or to non placental myometrium

The myometrial ^{133}Xe clearance in a case of intrauterine foetal death is illustrated by Fig 3 Two measurements in the same part of the uterine wall at 25 and 34 weeks of pregnancy in a diabetic woman gave the clearance values 22.4 and 33.4 ml/100 g/min, respectively The injections had apparently been into the myometrium at the placental site although this was not proved by arteriography In the 36th week of pregnancy signs of foetal death developed. The clearance examination was repeated at the same site and now the clearance rate had dropped to 9.4 ml/100

Table VI. ^{133}Xe Clearance in Myometrium (the Puerperium

Patient Name	Age Years	No. of Pregnancies	Days Post Partum	^{133}Xe Clearance		Mode of Injection
				Slope/Min (D)	Flow ml/100 g/min	
M.B.	28	9	0	0.121	19.5	transabdom.
G.B.O.	22	2	1	0.235	37.8	transabdom.
U.B.A.	20	1	1	0.164	26.4	transabdom.
A.A.	23	1	1	0.138	22.3	transabdom.
R.I.	32	5	2	0.267	43.0	transperit.
				0.240	38.6	laparotomy
C.E.	31	6	3	0.256	41.5	laparotomy
I.H.	41	1	3	0.080	12.9	transabdom.
H.O.	38	5	3	0.360	58.0	transabdom.
M.B.	25	1	3	0.126	20.2	transabdom.
A.R.	38	4	4	0.208	33.4	transperit.
				Mean ± SE	32.1 ± 3.99	

also. The highest recorded clearance rate, 58.0 ml/100 g/min, was found in a puerperal uterus on the third day after delivery

Comparison between g oups

Analysis of variance of the mean values of the four groups non-pregnant, early and midpregnant, late pregnant, and puerperal women is given in Table VII. It is clear that the null hypothesis that the four mean values are all equal can be rejected at the 1 per cent level of significance. Application of Scheffé s method of multiple comparisons shows that the mean myometrial ^{133}Xe clearance in the puerperium is significantly higher than that of both pregnant groups. The mean value in the non-pregnant group is not significantly different from any of the other groups. There is no statistically significant difference between the mean value in early and midpregnancy and that in late pregnancy

A comparison between the ^{133}Xe clearance of myometrium proper placental myometrium and non-defined myometrium (total series in table V) by the same methods (table VIII) shows that the differences between all three mean values are statistically significant ($P < 0.01$)

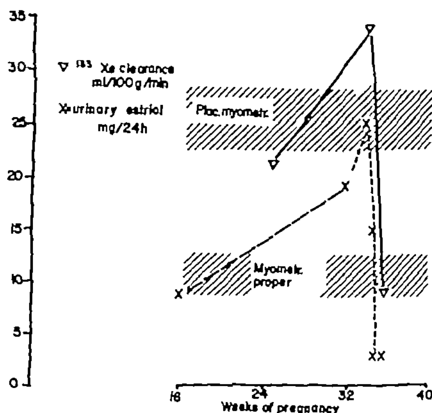


Fig. 3 Myometrial ^{133}Xe clearance and urinary oestriol excretion in a case of intrauterine foetal death in a diabetic woman. The shadowed areas represent the mean ^{133}Xe clearance ± 2 × standard error of the mean in placental and non-placental myometrium, respectively. The figures of the ordinate indicate both ^{133}Xe clearance in ml/100 g/min and urinary oestriol excretion in mg/24 h.

Table V. Mean Values of Myometrial ^{133}Xe Clearance in Pregnancy in Relation to Gestation Period and Placental Site

	^{133}Xe Clearance ml/100 g/min ± SE			
	Myometrium Proper	Placental Myometrium	Not Defined	Total
Early and midpregnancy	8.7 ± 1.73 (5)	25.3 ± 1.86 (8)	23.3 ± 1.48 (4)	19.9 ± 2.08 (17)
Late pregnancy	11.6 ± 1.08 (9)	26.3 ± 2.90 (8)	15.0 ± 2.24 (6)	17.6 ± 1.82 (23)
Total series	10.6 ± 0.97 (14)	25.8 ± 1.67 (16)	18.3 ± 1.95 (10)	18.6 ± 1.37 (40)

Figures in brackets indicate number of cases.

Table VIII

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares	f	Critical Value f .99
Between groups	1726.54	2	863.27	27.07	5.18
Within groups	1179.86	37	31.89		
Total	2906.40	39			

Discussion

^{133}Xe clearance is a measure of the capacity of the capillary blood flow to remove the injected isotope sometimes thought of as nutritional blood flow but better called effective capillary blood flow It must be understood that the distribution of this capillary blood flow may vary independently of the total regional blood flow since its regulation seems to be especially dominated by local metabolic factors (*cf e.g. Folkow* 1967) Factors influencing the clearance rate then must include the degree of vascularity of the tissue as well as, to some extent at least, the number of capillaries open to blood flow at any given moment.

The clearance method of estimating tissue blood flow has been criticized because of the variability of the clearance rate depending on, for example the site of injection and the composition of the tissue in which the tracer is deposited (*Assali* and *Morris* 1964) In the present study one major source of variability was controlled when the relation between the injection site and the placental implantation site was taken into account. Another point is of importance. Judging from the mono-exponential elimination curves obtained from the non-pregnant uterus, myometrium is a rather homogenous tissue Curves of a pronounced multi-exponential course suggests deposition in more than one type of tissue Thus only those experiments where the clearance curve was close to mono-exponential were accepted for calculation of blood flow The fairly good agreement between the results obtained at repeated observations in the same patient confirms the usefulness of the clearance method in this type of tissue.

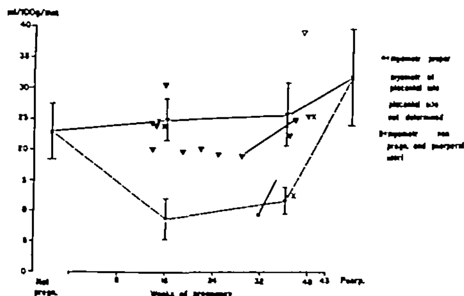


Fig. 4 ¹³³Xe clearance in non-pregnant, pregnant and puerperal myometrium. For non-pregnant and puerperal uteri only mean values are given. In the pregnant group open symbols represent individual values, filled symbols mean values. Vertical bars represent ± 2 × standard error of the mean. The figure illustrates the separation of myometrial ¹³³Xe clearance in pregnancy at two different levels representing different areas of the uterine wall placental and non-placental myometrium.

Table VII

Source of Variance	Sums of Squares	Degrees of Freedom	Mean Squares	f	Critical Value f .99
Between groups	1654 77	3	551.59	5.73	4 13
Within groups	5581 79	58	96.24		
Total	7236.56	61			

The results in non pregnant pregnant and puerperal women are collected in Fig 4 illustrating the seperation of myometrial ¹³³Xe clearance during pregnancy into two different levels, representing different areas of the uterine wall, myometrium at the placental site and non-placental myometrium.

placental and non-placental myometrium persists throughout the pregnancy

This finding, however is in contrast with the results of *Moore* and *Myerscough* (1957) who found the clearance rate of ^{24}Na to be significantly lower at the placental implantation site than in non-placental myometrium. These authors were unable to explain this unexpected finding. It is possible that the fact that sodium is not freely diffusible may be responsible for this difference in results. Particularly interesting is the observation that progesterone blocks sodium transport through the cell membrane (*Jung*, 1965) in view of *Csapo*'s theory (1959) that progesterone produced by the placenta diffuses directly into the neighbouring myometrium (*cf Bengtsson* 1967)

There is an increased vascularity at the placental implantation site but this may not be the sole explanation of the higher ^{133}Xe clearance rate at this location. The case described (Fig. 3) where the clearance rate at the placental site dropped to the level of non-placental myometrium when the foetus died, illustrates this. Again, a local effect of placental hormones on the vascular bed may be responsible The immediate fall in oestriol excretion coinciding with foetal death suggests that this hormone may be implicated. The vasodilating effect of oestrogens is well known. *Greiss* and *Marston* (1965) found that conjugated equine oestrogens caused a mean 36 per cent increase in uterine blood flow in pregnant ewes within two hours.

This effect of placenta on the local ^{133}Xe clearance rate in the myometrium must of course have a decisive effect on results obtained by this method In this selected series consisting of about equal numbers of observations on placental and non-placental myometrium, the a erage ^{133}Xe clearance rate was 18.6 ml/100 g/min as compared with 12.7 ml in the series of *Lysgaard* and *Lefèvre* (1965) These authors did not localize the placenta and all their experiments were performed in late pregnancy where the chance of hitting non-placental myometrium must be proportionately greater Thus, a low mean value is to be expected. The falling ^{133}Xe clearance rate during the second half of pregnancy found by *Guilhem et al.* (1965) may well have the same explanation.

When compared with ^{24}Na which has been used in most earlier studies (*Browne* and *Veall* 1953 *Morris et al* 1955 *Johnson* and *Clayton* 1957 *Moore* and *Myerscough* 1957 *Dixon et al* 1963) ^{133}Xe has the advantage of being chemically inert and freely diffusible through the cell membranes. Its gaseous nature soft gamma radiation and short half life (5 3 days) makes it convenient to handle and an ideal radioactive test substance in man.

The average ^{133}Xe clearance in myometrium of the non-pregnant uterus in the present series 23 ml/100 g/min, is the same as the mean value found by *Munck et al* (1964) with a transvaginal injection of the isotope in the isthmus uteri. Repeated observations in the same women showed no difference between the upper and lower segments of the uterine body There was however a wide scatter of the individual values. The only factor found to influence this variability was parity Although the group is too small to permit statistical analysis the observation that parous women had a myometrial ^{133}Xe clearance rate twice that of non-parous women is noteworthy By far the lowest clearance rate was found in a 17 year-old nulliparous girl with a small juvenile uterus. This is in agreement with the observation by *Horn* (1916) that the blood vessels of the parous uterus are larger and more abundant

The results in pregnant women confirm the finding by *Falk et al* (1967) that the myometrial ^{133}Xe clearance at the placental site is about twice that of non placental myometrium. *Falk et al.* made their experiments only in midpregnancy and calculated placental myometrial blood flow from the first rapid phase of elimination curves obtained in the vicinity of placenta and blood flow in myometrium proper both from the slow phase of these curves and from mono-exponential curves obtained at a distance from placenta The values found, however about 9 ml/100 g/min for myometrium proper and 21 8 ml/100 g/min for placental myometrium, are in close agreement with the mean values in early and midpregnancy in the present study 8 7 and 24.9 ml/100 g/min respectively In addition measurements in late pregnancy revealed approximately the same clearance rates 11 6 and 26 3 ml/100 g/min respectively showing that this difference between

between 500 and 700 ml/min is obtained (*Assali et al.* 1953 *Metcalfe et al* 1955) In view of the considerations given above these figures may be too low If a similar procedure is performed with the observed ^{133}Xe clearance values they should be multi plied only by the weight of the uterus, which is about 1 kg at term. The total myometrial blood flow at term then may be approximately 150–200 ml/min. The major part of the total uterine blood flow is destined for the spiral arteries, emptying their blood into the intervillous spaces. This fraction cannot be estimated by the clearance method, as there seems to be no means of puncturing the intervillous space with certainty (*Fuchs Spackman* and *Assali* 1963)

As pointed out earlier ^{133}Xe clearance reflects the effective capillary blood flow which is regulated by the local metabolic needs of the tissue. From this point of view the high ^{133}Xe clearance in puerperal myometrium is interesting. The mean value of 32.1 ml/100 g/min is in sharp contrast with the mean uterine blood flow found by *Assali et al.* (1953) using the nitrous oxide method, 9 ml/100 g/min. It is hard to explain this great differ ence as the figures should be comparable in the absence of foetus and placenta. The figure of Assali, however is based on 4 obser vations, 3 of which were immediately after caesarean section. There is reason to believe that the increased uterine tone immediately post partum diminishes the blood flow considerably The 11 observations in the present series were made 1–4 days after delivery on the intact uterus. The high clearance rate found may be explained by an increased capillary blood flow demanded by the intense metabolic processes involved in puerperal uterine in olution.

SUMMARY

1 ^{133}Xe clearance in the myometrium was studied in 11 non-pregnant women, in 40 pregnant women between the 10th and 43rd weeks of gestation, and in 10 women in the first few days of the puerperium. A total of 75 measurements were performed.

2. The mean ^{133}Xe clearance in non-pregnant myometrium was 23 0 ml/100 g/min. The wide scatter found (8.2–40.5 ml) could

In the present series there was no demonstrable difference in myometrial ^{133}Xe clearance at different periods of gestation, either at the placental site or in non placental myometrium. This is in agreement with the observation by *Assali et al.* (1960) who compiled their own results obtained by the nitrous oxide method with those of *Metcalfe et al.* (1955) and found that the blood flow per 100 g of pregnant uterus does not change significantly throughout the pregnancy. *Assali et al.* (1953, 1960) found a mean uterine blood flow at midpregnancy of 8.9 ml/100 g/min and 15 ml at term. The average value of *Metcalfe et al.* (1955) was 12.4 ml/100 g/min at term. These values cannot be compared with those obtained with the ^{133}Xe method as they constitute a measure of total uterine blood flow calculated per 100 g of uterus, placenta and foetus. They must also be regarded as minimal values.

Before the present investigation was started a pilot study on 8 cases of legal abortion and sterilization showed that the ^{133}Xe clearance in myometrium was greatly influenced by exposure of the pregnant uterus in the laparotomy wound. The first injection immediately after the abdomen was opened often gave a clearance rate around 15–25 ml/100 g/min. When the examination was repeated 20–30 minutes later figures as low as 4–5 ml/100 g/min were obtained from the same area. This coincided with evidence of increased myometrial tone, such as hardening and blanching of the uterine wall. Thus preliminary observation was in fact the reason for the technique used in the present study with transperitoneal injection of the isotope without exposing the uterus in the laparotomy wound. That uterine contractions reduce uterine blood flow is well known. *Ahlquist* (1950) found a decrease in uterine blood flow of 10–50 per cent with a rise in the uterine pressure of 20–30 mm Hg in dogs and *Borell, Fernström, Ohlson* and *Wiqvist* (1965) demonstrated with a cineangiographic technique that in women there is a slowing of the uterine blood flow with contractions. They attributed this finding to local compression of the arterioles by the myometrium.

If the values mentioned above, calculated by using the nitrous oxide method, are multiplied by an estimated weight of uterus and its contents of 4.5 kg, a total uterine blood flow at term of

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in part be explained by the differences in blood flow between parous and non parous uteri There was no difference between different parts of the uterine body

3 The mean myometrial ^{133}Xe clearance in pregnant uteri was 18 6 ml/100 g/min. The mean clearance rate in the myometrium at the placental implantation site was 25 8 ml/100 g/min and in non-placental myometrium 10 6 ml/100 g/min. This difference is statistically significant.

4 There was no difference in ^{133}Xe clearance rate between early and midpregnancy on one hand and late pregnancy on the other either with respect to placental or to non placental myometrium. This suggests that the effective capillary blood flow in the myometrium per unit weight of tissue is unchanged throughout the pregnancy

5 The highest mean ^{133}Xe myometrial clearance rate, 32.1 ml/100 g/min was found in the puerperium

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creased gradually suggesting constant resting muscle blood flow and rising skin blood flow during the second half of pregnancy In the present investigation the peripheral circulation has been studied with the same techniques in a group of pregnant diabetic women to detect possible deviations from normal pregnancy

^{133}Xe clearance has been shown to be a useful tool in studies of myometrial circulation during pregnancy (*Lysgaard* and *Lefevre* 1965 *Guilhem et al.* 1965 *Falk et al.* 1967 *Jansson* 1969) With this technique it was demonstrated that the myometrial blood flow at the placental bed is about twice that of non-placental myometrium (*Falk et al.* 1967 *Jansson* 1969) and that the myometrial blood flow per unit weight of tissue is largely unchanged throughout pregnancy (*Jansson* 1969) Thus, another purpose of this investigation was to study the ^{133}Xe clearance of the myometrium in diabetic pregnancy and compare the results with those previously obtained in normal pregnancy

Material

Twenty-one pregnant women with diabetes mellitus were studied. Relevant clinical data on each patient are given in Table I. The severity of the diabetes was classified according to *White* (1949) with the modifications used by *Pedersen* (1967) *I.e* Class A diabetes treated by diet alone Class B insulin treated diabetes diagnosed at the age of 20 or later and without late diabetic complications Class C diabetes diagnosed before 20 years of age and without late diabetic complications Class D diabetes with non-proliferative retinopathy Class F diabetes with vascular nephropathy and/or proliferative retinopathy Class E (calcification of pelvic arteries) was not used and there were no A and F-cases in the series studied. Prognostically bad signs during the present pregnancy (PBSP) according to *Pedersen* and *Pedersen* (1965) are also noted in the table The patients were treated according to the now widely accepted principles with repeated admissions to hospital and an attempt throughout pregnancy to keep the blood glucose level as close to normal as possible to avoid keto-acidosis The time for and the mode of delivery was chosen in each individual case on the basis of the severity of the

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FOREARM AND MYOMETRIAL BLOOD FLOW IN DIABETIC PREGNANCY STUDIED BY VENOUS OCCLUSION PLETHYSMOGRAPHY AND ^{133}XENON CLEARANCE

BY

INGE JANSSON

When pregnancy is complicated by diabetes mellitus the outcome of the pregnancy is decided to a great extent by the degree of maternal vascular disease (*cf* e g *White* 1949 1958 *Pedersen* 1967) That the state of the vessels may interfere with the normally occurring circulatory changes during pregnancy is obvious. In spite of this fact very few studies of the circulation in diabetic pregnancy have been performed.

Spetz (1965) studied the peripheral circulation in diabetic pregnancy by means of venous occlusion plethysmography of the forearm and found a progressive increase of the same magnitude as during normal pregnancy *Brudenell Miles* and *Coleman* (1961) used the clearance rate of radioactive sodium (^{24}Na) from the uterine wall as an indicator of myometrial blood flow in diabetic pregnancy but were not able to demonstrate any difference from normal pregnancy

^{133}Xenon has certain advantages over ^{24}Na in measurements of local tissue blood flow (*Lassen et al* 1964) and can be used to evaluate the blood flow selectively in different tissue compartments. *Spet* and *Jansson* (1969) found the ^{133}Xe clearance in forearm muscle to be unchanged during normal pregnancy while the forearm blood flow recorded by the plethysmograph in-

Table I. Continued

	Age Years	Preg-nancy No.	White Class	PBCP	Hydram-nios	Week of Delivery	Mode of Delivery	Infant
	19	1	C	0	0	39	Induction + VE	♂ 3310 g cong. heart disease?
	22	1	B	0	0	40	Induction + VE	♂ 3390 g normal
	22	1	B	0	0	40	N.D	♂ 4180 g normal

bbreviations

ISP prognostically bad signs in pregnancy (*Pedersen* and *Pedersen*, 1965) toxaemia, keto-acidosis, neglectors (patients neglecting proper diabetic control during pregnancy pyelo-nephritis

ND normal delivery in OA position. VE = vacuum extraction.

RDS idiopathic respiratory distress syndrome.

disease, the obstetrical history the course of the pregnancy and daily determinations of urinary oestriol excretion.

Methods

To obtain best possible basal conditions the patients rested in bed for about 30 minutes before any measurements were made. The room temperature was maintained at 22 C The ^{133}Xe muscle clearance measurement was always performed before the plethysmographic examination. Both methods of estimating peripheral blood flow were employed 2–4 times during the course of the pregnancy The results were compared with those previously obtained in healthy non-pregnant and pregnant women (*Spetz* and *Jansson* 1969)

1 ^{133}*Xe clearance in forearm muscle* The isotope was injected into the extensor musculature of the forearm and the clearance rate recorded as described in detail previously (*Spetz* and *Jansson* 1969) The local muscle blood flow (MBF) was calculated from the slope of the elimination curve (D) according to the

Table I. *Clinical Data*

Case No.	Age Years	Pregnancy No.	White's Class	PBSP	Hydramnios	Week of Delivery	Mode of Delivery	Infant
1	18	1	C	tox.	0	37	Induction + VE	♂ 3700 g neonat. death
2	25	1	C	0	0	38	Induction + N.D	♀ 2400 g normal
3	36	4	B	acidosis negl	0	35	N.D	♀ 3320 g dysmature
4	27	1	B	0	0	37	Induction + VE	♀ 3000 g neonat. death IRDS
5	20	1	C	0	+	36	N.D	♀ 2950 g normal
6	23	4	D	0	0	38	Caesarean section	♀ 2640 g normal
7	29	1	D	tox.	0	37	N.D	♂ 2930 g Intrauterine death
8	24	2	C	0	0	38	Induction + N.D	♂ 3970 g normal
9	21	1	C	0	0	36	Caesarean section	♂ 3780 g normal
10	27	5	C	acidosis	0	33	N.D	♂ 1730 g multiple malform.
11	26	1	B	0	+	37	Caesarean section	♂ 2850 g normal
12	35	2	D	0	+	38	Caesarean section	♀ 3410 g normal
13	18	2	D	0	+	39	Caesarean section	♂ 2550 g dysmature
14	23	1	C	0	0	39	Induction + N.D	♀ 2580 g normal
15	31	2	C	0	+	38	Induction + N.D	♂ 4530 g dead at 3 m. brain tumor
16	28	2	D	0	0	38	Caesarean section	♀ 3790 g normal
17	23	1	D	0	0	39	Caesarean section	♂ 3400 g normal
18	19	1	C	pyelonephr	+ +	38	Spont. breech	♂ 3940 g normal

Table I. Continued

Age Years	Preg-nancy No.	White's Class	PBCP	Hydram-nios	Week of Delivery	Mode of Delivery	Infant
19	1	C	0	0	39	Induction + VE	♂ 3310 g cong. heart disease?
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Abbreviations

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ND normal delivery in OA position. VE = vacuum extraction.

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disease the obstetrical history the course of the pregnancy and daily determinations of urinary oestriol excretion.

Methods

To obtain best possible basal conditions the patients rested in bed for about 30 minutes before any measurements were made. The room temperature was maintained at 22 C. The ^{133}Xe muscle clearance measurement was always performed before the plethysmographic examination. Both methods of estimating peripheral blood flow were employed 2–4 times during the course of the pregnancy. The results were compared with those previously obtained in healthy non-pregnant and pregnant women (*Spetz* and *Jansson* 1969).

1. ^{133}*Xe clearance in forearm muscle.* The isotope was injected into the extensor musculature of the forearm and the clearance rate recorded as described in detail previously (*Spetz* and *Jansson* 1969). The local muscle blood flow (MBF) was calculated from the slope of the elimination curve (D) according to the

equation MBF = D 161 ml per 100 g of tissue per minute (*Lassen et al.* 1964)

2. *Plethysmography* The blood flow in the right forearm was measured by venous occlusion plethysmography in the same manner as described by *Spet-* (1964) The water temperature of the plethysmograph was always maintained at 34 C The mean of at least ten measurements was taken as the total forearm blood flow at rest, expressed in ml per 100 ml of tissue per minute.

3 ^{133}*Xe clearance in myometrium.* The technique used has been described in detail before (*Jansson* 1969) In most cases the ^{133}Xe solution was injected transabdominally into the anterior uterine wall without knowledge of the placental site. In two patients (no 13 and 17) the placental site was determined at Caesarean section in one (10) by pelvic arteriography and in another one (14) by isotope (^{99m}Tc) scanning In four additional cases (12, 18 19 20) the placenta could be located with the aid of a Doptone ultrasonic foetal pulse detector (Smith Kline Instrument Co England) *Bishop* (1966) has shown that the placental site can be determined with this instrument in about 85 per cent of cases. Whenever possible the ^{133}Xe clearance examination was repeated at varying intervals during the pregnancy and always in approximately the same area of the uterine wall after allowing for the uterine growth. As the myometrial blood flow per unit tissue is largely unchanged throughout pregnancy a mean value was calculated for those patients where more than one examination was made

Statistics. Mean values standard error of the mean (SE) and regression lines were calculated by conventional statistical methods In the text and tables mean values are given ± SE. The significance of differences between groups was tested by the *t* test. With unequal variances the Welch procedure was applied

The reproducibility of the ^{133}Xe clearance method when applied to myometrium was tested by analysis of variance showing a significantly smaller variation ($P < 0.001$) in the same patient than between patients Furthermore a test of squared differences between the first and second measurements showed that the difference between two measurements in the same patient was not significantly different from zero

The statistical formulas used were taken from *Brownlee* (1965). When not otherwise stated the tests of significance of differences were performed at the 5 per cent level.

Results

Peripheral blood flow

The results of 23 determinations of forearm blood flow by plethysmography and 30 measurements of ^{133}Xe muscle clearance in 16 pregnant diabetics are given in Table II. Five of these cases were examined only once, the rest two to four times during the course of the pregnancy. The mean total forearm blood flow at rest for the whole group of pregnant diabetic women, 3.9 ± 0.48 ml/100 ml/min, is significantly higher than the mean resting forearm blood flow of non-pregnant healthy women, 2.5 ± 0.32 ml/100 ml/min. The mean ^{133}Xe clearance in forearm muscle in the pregnant diabetics, 3.3 ± 0.25 ml/100 g/min, however, is not significantly different from that of normal non-pregnant women 3.2 ± 0.22 ml/100 g/min nor from that of normal pregnant women 3.6 ± 0.24 ml/100 g/min.

The mean values for total forearm blood flow and ^{133}Xe muscle clearance at different stages of pregnancy are given in Table III. The mean total forearm blood flow in the period 13–24 weeks of pregnancy, 2.9 ± 0.50 ml/100 ml/min, is not significantly different from that of non-pregnant healthy subjects. The differences between the mean values of forearm blood flow in the periods 25–32 and 33–37 weeks of gestation, 3.8 ± 0.71 and 5.8 ± 0.99 ml/100 ml/min, respectively, and that of the non-pregnant group, however, are stastically significant. None of the mean values of ^{133}Xe muscle clearance in the diabetics at the different stages of pregnancy is significantly different from that in non-pregnant women.

Fig. 1 illustrates that the total forearm blood flow increased progressively from midpregnancy until 37 weeks while the forearm muscle blood flow as judged from ^{133}Xe clearance remained unchanged throughout the pregnancy. As the diabetic pregnancies were interrupted before term no values were obtained after 37 weeks.

In Fig. 2 the individual total forearm blood flow values are

Table II. *Forearm Blood Flow as Determined by Venous Occlusion Plethysmography and by ¹³³Xe Muscle Clearance in Pregnancy Complicated by Diabetes Mellitus*

Case No.	Week of Pregnancy	Blood Flow Plethysm. ml/100 ml/min ± SE	¹³³Xe Clearance	
			Slope/min (D)	Flow ml/100 g/min
1	28		0.018	2.9
	32	1 6 ± 0.04	0.010	1.6
	37	1 8 ± 0.07	0.016	2.6
2	24		0.010	1.6
	31	4.6 ± 0.16	0.016	2.6
	35	3.7 ± 0 16	0.021	3.4
	37	8.5 ± 0.22	0.049	7.8
3	34	3.7 ± 0 12	0.012	2.0
4	32		0.021	3 4
5	31	2.0 ± 0.07	0.017	2.7
	35	4.8 ± 0.19	0.023	3.7
6	20	1.6 ± 0.05	0.025	4.0
	21	2.7 ± 0.06	0.027	4 4
	31	5.9 ± 0.35	0.036	5.8
	35	9.5 ± 0.36	0.026	4 2
	37		0.018	2.9
7	25	2.6 ± 0 16	0.015	2.4
	34	7 7 ± 0.36	0.036	5.8
8	20	4.3 ± 0.14	0.023	3.7
	31	7 4 ± 0.37	0 019	3.3
	34	7.3 ± 0.28	0.023	3.7
9	22	2.8 ± 0.18	0.018	2.9
	31	3.4 ± 0.26	0.024	3.9
10	29	2.3 ± 0.09	0.023	3.7
	32	4 0 ± 0 16	0.029	4.7
11	18	2.7 ± 0.06		
	23	1 7 ± 0.04		
12	13		0.020	3.2
13	36		0.012	1.9
15	35		0.015	2.4
16	14		0.016	2.6
21	26		0.034	5.5
Mean ± SE		3 9 ± 0.48		3.3 ± 0.25

The group mean values are calculated on mean values for each case.

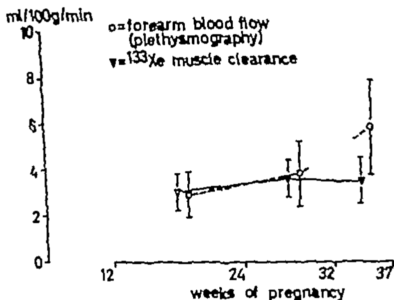


Fig. 5. Forearm blood flow determined by plethysmography and ^{133}Xe clearance in forearm muscle at different stages of pregnancy complicated by diabetes mellitus. Mean values are given ± 2 SE.

Table III. Forearm Blood Flow as Determined by Plethysmography and ^{133}Xe Muscle Clearance at Different Stages of Pregnancy in Women with Diabetes Mellitus

Mean Values ± SE   n = Number of Cases.

Method	Weeks of Pregnancy		
	13–24	25–32	33–37
Plethysmography ml/100 ml/min	2.9 ± 0.50 n = 4	3.8 ± 0.71 n = 8	5.8 ± 0.99 n = 7
^{133}Xe clearance ml/100 g min	3.0 ± 0.37 n = 6	3.6 ± 0.39 n = 10	3.5 ± 0.48 n = 9

Table II. *Forearm Blood Flow as Determined by Venous Occlusion Plethysmography and by ^{133}Xe Muscle Clearance in Pregnancy Complicated by Diabetes Mellitus*

Case No.	Week of Pregnancy	Blood Flow Plethysm. ml/100 ml/min ± SE	^{133}Xe Clearance	
			Slope/min (D)	Flow ml/100 g/min
1	28		0.018	2.9
	32	1.6 ± 0.04	0.010	1.6
	37	1.8 ± 0.07	0.016	2.6
2	24		0.010	1.6
	31	4.6 ± 0.16	0.016	2.6
	35	3.7 ± 0.16	0.021	3.4
	37	8.5 ± 0.22	0.049	7.8
3	34	3.7 ± 0.12	0.012	2.0
4	32		0.021	3.4
5	31	2.0 ± 0.07	0.017	2.7
	35	4.8 ± 0.19	0.023	3.7
6	20	1.6 ± 0.05	0.025	4.0
	21	2.7 ± 0.06	0.027	4.4
	31	5.9 ± 0.35	0.036	5.8
	35	9.5 ± 0.36	0.026	4.2
	37		0.018	2.9
7	25	2.6 ± 0.16	0.015	2.4
	34	7.7 ± 0.36	0.036	5.8
8	20	4.3 ± 0.14	0.023	3.7
	31	7.4 ± 0.37	0.019	3.3
	34	7.3 ± 0.28	0.023	3.7
9	22	2.8 ± 0.18	0.018	2.9
	31	3.4 ± 0.26	0.024	3.9
10	29	2.3 ± 0.09	0.023	3.7
	32	4.0 ± 0.16	0.029	4.7
11	18	2.7 ± 0.06		
	23	1.7 ± 0.04		
12	13		0.020	3.2
13	36		0.012	1.9
15	35		0.015	2.4
16	14		0.016	2.6
21	26		0.034	5.5
Mean ± SE		3.9 ± 0.48		3.3 ± 0.25

The group mean values are calculated on mean values for each case

Table IV ^{133}Xe Clearance in Myometrium in Pregnancy Complicated by Diabetes Mellitus

Case No.	Week of Pregnancy	^{133}Xe Clearance Slope/min (D)	Flow ml/100 g/min	Mean	Site of Injection
1	28	0.042	6.8		Not defined
	32	0.055	8.9	9.5	
	37	0.080	12.9		
2	24	0.168	27.0		Not defined
	31	0.152	24.4	27.3	
	37	0.190	30.6		
3	34	0.090	14.5		Not defined
4	32	0.094	15.1		Not defined
5	31	0.083	14.1		Not defined
6	20	0.218	35.0		Not defined
	31	0.192	30.9	30.3	
	37	0.155	25.0		
7	25	0.129	21.2		Not defined
	34	0.210	33.8	27.5	
	36	0.057	9.2		
8	20	0.142	22.8		Not defined
	31	0.124	20.0	21.8	
	34	0.140	22.6		
9	22	0.115	18.5	21.5	Not defined
	31	0.152	24.4		
10	29	0.226	36.4	33.5	Placental myometr
	32	0.190	30.6		
12	33	0.182	29.3	30.8	Placental myometr
	36	0.200	32.2		
13	36	0.113	18.2		Placental myometr
14	36	0.110	17.7		Placental myometr
17	39	0.090	14.5		Non-placental myometr
18	33	0.060	9.7		Non-placental myometr
19	32	0.155	25.0		Placental myometr
	35	0.127	20.5	24.4	
	39	0.172	27.7		
20	31	0.147	23.6	21.1	Placental myometr
	35	0.115	18.5		
21	32	0.058	9.3	13.9	Not defined
	37	0.114	18.4		
Mean ± SE				20.3 ± 1.73	

This value was obtained after intrauterine death of the foetus and is not included in the mean value

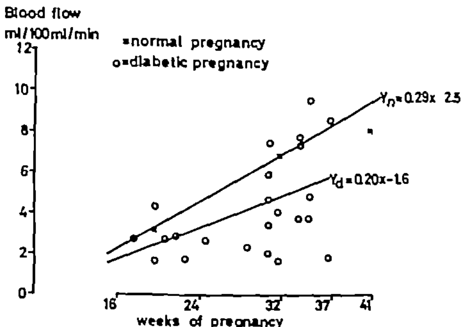


Fig. 2. Relation of forearm blood flow determined by plethysmograph to duration of pregnancy in normal pregnancy and in pregnancy complicated by diabetes mellitus.

plotted against the duration of pregnancy For comparison the blood flow values for normal pregnant women where the same plethysmographic water temperature (34 C) was used, are given. The calculated regression lines for diabetic pregnancy between 16 and 37 weeks $Y_d = 4.2 + 0.20(x - 29)$ and for normal pregnancy between 16 and 41 weeks $Y = 6.8 + 0.29(x - 32)$ show that the forearm blood flow increased in a linear manner during the time intervals studied The regression coefficients are not significantly different from each other but the lines are not identical ($P < 0.05$) This means that the total forearm blood flow in the group of pregnant diabetic women increased at the same rate as in normal pregnancy but that the mean forearm blood flow was significantly lower in the pregnant diabetics

^{133}Xe clearance in myometrium

Thirty five technically acceptable measurements were performed in 18 diabetic patients at different periods of gestation (Table IV) The mean ^{133}Xe clearance in myometrium regardless of the

Table IV ^{133}Xe Clearance in Myometrium in Pregnancy Complicated by Diabetes Mellitus

Case No.	Week of Pregnancy	^{133}Xe Clearance			Site of Injection
		Slope/min (D)	Flow ml/100 g/min	Mean	
1	28	0.042	6.8		Not defined
	32	0.055	8.9	9.5	
	37	0.080	12.9		
2	24	0.168	27.0		Not defined
	31	0.152	24.4	27.3	
	37	0.190	30.6		
3	34	0.090	14.5		Not defined
4	32	0.094	15.1		Not defined
5	31	0.088	14.1		Not defined
6	20	0.218	35.0		Not defined
	31	0.192	30.9	30.3	
	37	0.155	25.0		
7	23	0.129	21.2		Not defined
	34	0.210	33.8	27.5	
	36	0.057	9.2		
8	20	0.142	22.8		Not defined
	31	0.124	20.0	21.8	
	34	0.140	22.6		
9	22	0.115	18.5		Not defined
	31	0.152	24.4	21.5	
10	29	0.226	36.4		Placental myometr
	32	0.190	30.6	33.5	
12	33	0.182	29.3		Placental myometr.
	36	0.200	32.2	30.8	
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14	36	0.110	17.7		Placental myometr
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	35	0.127	20.5	24.4	
	38	0.172	27.7		
20	31	0.147	23.6		Placental myometr
	35	0.115	18.5	21.1	
21	32	0.058	9.3		Not defined
	37	0.114	18.4	13.9	
Mean ± SE				20.3 ± 1.73	

This value was obtained after intrauterine death of the foetus and is not included in the mean value

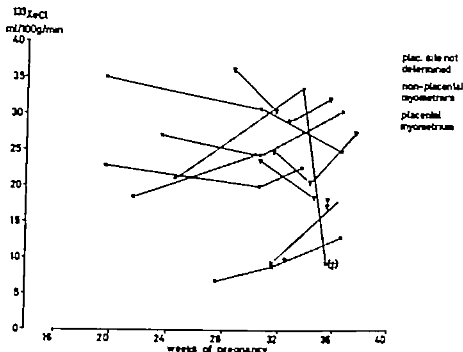


Fig. 3. ^{133}Xe clearance in myometrium in pregnancy complicated by diabetes mellitus. The lines connect clearance values obtained at different stages of pregnancy in the same patients. The shadowed areas represent mean values of ^{133}Xe clearance in placental and non-placental myometrium in normal preg nancy ± 3 SE. The value marked with a cross was obtained after intrauterine death of the foetus.

placental site was 20.3 ± 1 73 ml/100 g/min. This is not significantly different from the corresponding mean value of normal pregnancy 18 6 ± 1.37 ml/100 g/min. The mean ^{133}Xe clearance in six cases where the ^{133}Xe injection was made at the placental implantation site was 24.3 ± 2.69 ml/100 g/min. The correspond ing mean value in normal pregnancy was 25 8 ± 1 67 ml/100 g/min. The difference is not statistically significant. Only in two cases could it be determined that the ^{133}Xe injection was made in non placental myometrium. The mean value 12 1 ml/100 g/min, is about equal to the corresponding mean ^{133}Xe clearance in normal pregnancy 10 6 ml/100 g/min.

It is rather obvious from the graphic representation of the individual ^{133}Xe myometrial clearance values in Fig 3 that there was

no essential difference between different stages of pregnancy. The figure also illustrates the tendency of the clearance values to fall into two different groups representing placental and non-placental myometrium. The steep fall in ^{133}Xe clearance after intrauterine death of the foetus in Case no. 7 has been commented on earlier (*Jansson 1969*).

No difference in the myometrial ^{133}Xe clearance rate between cases with severe and those with mild diabetes or those with and without PBSP could be detected, nor was there any appreciable difference between cases with and without hydramnios.

Discussion

Diabetes mellitus is characterized by early and frequent occurrence of vascular lesions, which are of two types: atherosclerosis which is especially prone to involve small vessels and the so called microangiopathy distinguished by thickening of the capillary basement membrane (for references see *Warren*, *LeCompte* and *Legg*, 1966). The lesions have been demonstrated in skeletal muscle as well as in the skin.

In non-pregnant diabetics a decreased peripheral circulation, especially in the skin of the toes and fingers, has been observed with various techniques (*Mendlowitz*, *Grossman* and *Alpert* 1953, *Meglbow*, *Meglbow*, *Pollack*, *Bookman* and *Osserman* 1953, *Báróny* 1955, *Sigroth* 1957). *Weber* and *Wicht* (1962) in a combined plethysmographic and histopathological study were able to correlate the abnormal distal circulation in diabetics with the occurrence of the above mentioned vascular lesions in the skin.

When diabetes is complicated by pregnancy the increasing peripheral blood flow will in some patients take place in a damaged vascular bed. In the present study it was shown that the peripheral blood flow measured by forearm plethysmography increased from an average of 2.9 ml in the period 16–24 weeks of pregnancy to 5.8 ml/100 ml/min at 33–37 weeks as compared to 3.5 and 7.6 ml/100 ml/min, respectively, in normal pregnancy. The forearm blood flow increased at the same rate in the pregnant diabetics as in normal pregnancy but their mean forearm

blood flow was significantly lower As can be seen from Fig. 2, however there was a wide scatter of the individual values, some falling within the normal range This is in agreement with the fact that only some of the patients in the age group studied may be expected to show vascular lesions severe enough to influence the rate of blood flow The low values were often recorded in cases with so called prognostically bad signs present (PBSP i.e. toxaemia acidosis etc as in Cases no 1 3 and 10) but were also found in those without (no 9) In this small and rather uniform series the possible influence of the severity and the duration of the disease as judged by White s classification could not be assessed

The results are somewhat at variance with those obtained by *Spet~* (1965) In a consecutive study of 13 diabetic pregnant women he found that the increase of forearm blood flow was of the same magnitude as in normal pregnancy but that the maximal values were reached already at 29–31 weeks of pregnancy whereafter a gradual decline took place In the present series no such early peak was found This discrepancy however may be because the severity of the disease in the individual cases may not be comparable in these small series. Furthermore *Spet~* used a plethysmographic water temperature of 36 C which tends to give a higher average forearm blood flow by vasodilatation in the skin as compared with 34 C used in the present study

As in normal pregnancy the ^{133}Xe clearance of forearm muscle in the diabetic women did not show any significant change during the course of pregnancy The mean ^{133}Xe muscle clearance at rest, 3 3 ml/100 g/min, was not significantly different from that of normal pregnant women, nor in fact from that of non preg nant healthy women ^{133}Xe clearance can be assumed to be a measure of the effective capillary blood flow of skeletal muscle which accordingly does not seem to be altered during diabetic pregnancy This is in principle in agreement with the results of *Munck Lindbjerg, Binder Lassen* and *Trap-Jensen* (1966) who studied muscle blood flow with ^{133}Xe clearance technique in non pregnant diabetics and found no difference in resting blood flow or maximal blood flow capacity as compared with non-diabetics

As ^{133}Xe is freely diffusible its clearance rate is limited only by the rate of capillary blood flow and not by the state of the capillary membrane. *Trap-Jensen*, *Alpert*, *del Rio* and *Lassen* (1967) found an increased capillary diffusion capacity for sodium (^{24}Na) in diabetics, which they attributed to the capillary angiopathy. No similar studies during diabetic pregnancy are available. *Spetz* (1965), however, found a decreased capillary filtration capacity as measured by plethysmography in diabetic pregnancy, suggesting a disturbed function of the capillary membrane.

In the second half of both normal and diabetic pregnancy the forearm blood flow as recorded with a plethysmograph increased gradually, while the muscle blood flow as judged from ^{133}Xe clearance remained constant. The interpretation of this finding is not as simple as it may seem. It may be explained by the assumption that in those pregnant diabetic cases where a reduced peripheral blood flow is observed, this reduction takes place essentially in the skin. As has been discussed earlier (*Spetz* and *Jansson* 1969) such a conclusion implies a distribution of peripheral blood flow in late pregnancy which cannot be accepted unreservedly. The question needs further elucidation.

The really important problem in diabetic pregnancy is how the disease affects the foeto-placental unit. The high foetal mortality in connection with advanced vascular disease in diabetes has led to the assumption that there is an inadequate uteroplacental blood flow (*cf. White* 1949, 1965). In the present study the average myometrial capillary blood flow in diabetic pregnancy as measured by ^{133}Xe clearance, 20.3 ml/100 g/min, was not significantly different from that of normal pregnancy, 18.6 ml. The same is true for ^{133}Xe clearance in the myometrium at the placental implantation site, which in diabetic pregnancy was found to be 24.3 ml, and for myometrium at a distance from placenta, the ^{133}Xe clearance in which was 12.1 ml/100 g/min.

Considering the influence of the placenta on the myometrial circulation, it is unfortunate that the placental site could be determined only in a limited number of the cases investigated. This disadvantage, however, was to a certain extent eliminated by the fact that the ^{133}Xe clearance measurements were repeated in the same area of the uterine wall, making the placental influ-

blood flow was significantly lower As can be seen from Fig. 2, however there was a wide scatter of the individual values some falling within the normal range. This is in agreement with the fact that only some of the patients in the age group studied may be expected to show vascular lesions severe enough to influence the rate of blood flow The low values were often recorded in cases with so called prognostically bad signs present (PBSP *i.e.* toxaemia acidosis etc. as in Cases no 1 3 and 10) but were also found in those without (no 9) In this small and rather uniform series the possible influence of the severity and the duration of the disease as judged by White s classification could not be assessed

The results are somewhat at variance with those obtained by *Spetz* (1965) In a consecutive study of 13 diabetic pregnant women he found that the increase of forearm blood flow was of the same magnitude as in normal pregnancy but that the maximal values were reached already at 29–31 weeks of pregnancy whereafter a gradual decline took place In the present series no such early peak was found. This discrepancy however may be because the severity of the disease in the individual cases may not be comparable in these small series Furthermore *Spetz* used a plethysmographic water temperature of 36 C which tends to give a higher average forearm blood flow by vasodilatation in the skin as compared with 34 C used in the present study

As in normal pregnancy the ^{133}Xe clearance of forearm muscle in the diabetic women did not show any significant change during the course of pregnancy The mean ^{133}Xe muscle clearance at rest, 3 3 ml/100 g/min was not significantly different from that of normal pregnant women nor in fact from that of non-pregnant healthy women ^{133}Xe clearance can be assumed to be a measure of the effective capillary blood flow of skeletal muscle which accordingly does not seem to be altered during diabetic pregnancy This is in principle in agreement with the results of *Munck Lindbjerg, Binder Lassen* and *Trap-Jensen* (1966) who studied muscle blood flow with ^{133}Xe clearance technique in non pregnant diabetics and found no difference in resting blood flow or maximal blood flow capacity as compared with non-diabetics

ever is also influenced by the state of the placental vessels While some investigators have demonstrated the same vascular changes in diabetic placentas as in other tissues, others have not been able to confirm their findings (*cf* *Warren et al.* 1966) *Berglund* and *Zetterström* (1954) found a low oxygen content in umbilical venous blood in babies of diabetic mothers at Caesarean section, while *Peel* (1962) in studying oxygen content and saturation of cord blood and hemoglobin levels at birth found no evidence that placental insufficiency and foetal hypoxia was an important cause of intrauterine foetal death in diabetic pregnancy

SUMMARY

1 In 16 pregnancies complicated by diabetes mellitus repeated measurements of resting forearm blood flow by plethysmography and of forearm muscle blood flow by ^{133}Xe clearance technique were performed between 13 and 37 weeks of gestation.

2 The myometrial circulation was evaluated in 18 pregnant diabetic women by consecutive measurements of ^{133}Xe myometrial clearance between 20 and 39 weeks of pregnancy

3 The total forearm blood flow at rest increased at the same rate in diabetic as in normal pregnancy The average forearm blood flow however was significantly lower in the diabetic women. The capillary muscle blood flow measured by ^{133}Xe clearance on the other hand, was of the same magnitude as in normal pregnancy and in healthy non-pregnant women and it remained constant during the course of pregnancy The significance f these observations is discussed.

4 The myometrial blood flow as judged from ^{133}Xe clearance was not significantly different from the values found in normal pregnancy either at the placental site or in non-placental myometrium The capillary myometrial blood flow per unit weight of tissue was unchanged throughout pregnancy

Acknowledgements

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ence relatively constant from one examination to another in the individual patient. From Fig 3 it can also be seen that the ^{133}Xe clearance values in cases of undetermined placental site have a tendency to be distributed at two different levels, probably representing placental and non placental myometrium. Most values fall within the range of placental myometrium and no value below the range of non placental myometrium which makes it highly probable that the myometrial blood flow in diabetic pregnancy is at least not lower than in normal pregnancy As in normal pregnancy no change in myometrial blood flow per unit weight of tissue during the course of pregnany could be demonstrated.

The results are a confirmation of those of *Brudenell et al.* (1961) who in a study of 68 pregnant diabetic women with ^{24}Na clearance were not able to detect any difference in myometrial circulation between these and a group of normal pregnant women. They did, however find a shorter ^{24}Na clearance time in cases which subsequently resulted in foetal death than in those cases where the baby survived. The authors could not explain this unexpected finding. In view of the above mentioned observation of *Trap-Jensen et al.* (1967) the short ^{24}Na clearance time may perhaps be explained by an increased sodium diffusion capacity indicating advanced capillary damage in the myometrium in association with foetal death.

Histopathological studies of the myometrium in diabetes seem to be very few While *White* (1965) claims that the small myometrial blood vessels age very rapidly in diabetes *Pinkerton* (1963) found narrowing of the arterioles in the placental bed in only 3 out of 54 diabetic mothers. The normal myometrial circulation in the placental bed found in the present study agrees with the latter observation.

It must be remembered that ^{133}Xe clearance gives a measure only of myometrial circulation. The major and most important part of uterine blood flow is directed via the spiral arteries to the chorio-decidual space. This fraction may be reduced in diabetic pregnancy in spite of a normal myometrial blood flow although it is reasonable to assume that parallel changes occur in the two vascular systems The oxygen supply to the foetus how

Hibbard, Bryan M and Hibbard, Elizabeth D Acta obst. et gynec. scandinav 48, 339, 1969

From the Department of Obstetric and Gynaecology (Professor T N A. Jeff oate M.D) University of Liverpool England

THE PROPHYLAXIS OF FOLATE DEFICIENCY IN PREGNANCY

BY

BRYAN M. HIBBARD AND ELIZABETH D. HIBBARD

Increasing awareness of the frequency of folate deficiency in pregnancy and of its relationship with pregnancy complications such as abortion and abruptio placentae (*Hibbard* 1964) as well as with megaloblastic anaemia, has led to the extensive administration of folic acid supplements to pregnant women

Until recently the commonly used dosage has been 5 mg given 1–3 times daily Such dosage is almost certainly unnecessarily large It may be dangerous if administered to patients with unrecognised Vitamin B_{12} deficiency although this very slight risk has probably been over-emphasized in the past.

Recent trials have been designed to determine suitable prophylactic dosage in the microgramme range Thus *Chanarin* and his colleagues (1965) in London found that a supplement of 20 μg/day was insufficient to prevent megaloblastic anaemia but subsequently found that in the majority of women 100 μg was an adequate supplement, as judged by reticulocyte count and serum folate levels (*Chanarin* et al., 1968) *Hansen* and *Rybo* (1967) also found that a supplement of 100 μg was adequate. *Willoughby* and *Jewell* (1966) investigating a population of poorer social and nutritional status in Glasgow suggested that supplementation in the order of 300 μg folic acid daily is desirable *Chisholm* (1966) found no significant difference in folate status between patients given supplements of 5 mg and 500 μg.

Two particular problems arise in the conduct of such trials. The first, commented on by *Chisholm*, is the difficulty in assessing the value of prophylactic therapy in a population with little evidence

and the Medical Society of Goteborg The statistical analysis was performed by Mrs Gull Britt Palm fil. kand.

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THE PROPHYLAXIS OF FOLATE DEFICIENCY IN PREGNANCY

BY

BRYAN M. HIBBARD AND ELIZABETH D. HIBBARD

Increasing awareness of the frequency of folate deficiency in pregnancy and of its relationship with pregnancy complications such as abortion and abruptio placentae (*Hibbard* 1964) as well as with megaloblastic anaemia, has led to the extensive administration of folic acid supplements to pregnant women.

Until recently the commonly used dosage has been 5 mg given 1 3 times daily. Such dosage is almost certainly unnecessarily large. It may be dangerous if administered to patients with unrecognised Vitamin B_{12} deficiency although this very slight risk has probably been over-emphasized in the past.

Recent trials have been designed to determine suitable prophylactic dosage in the microgramme range. Thus *Chanarin* and his colleagues (1965) in London found that a supplement of 20 μg/day was insufficient to prevent megaloblastic anaemia, but subsequently found that in the majority of women 100 μg was an adequate supplement, as judged by reticulocyte count and serum folate levels (*Chanarin* et al., 1968). *Hansen* and *Rybo* (1967) also found that a supplement of 100 μg was adequate. *Willoughby* and *Jewell* (1966) investigating a population of poorer social and nutritional status in Glasgow suggested that supplementation in the order of 300 μg folic acid daily is desirable. *Chisholm* (1966) found no significant difference in folate status between patients given supplements of 5 mg and 500 μg.

Two particular problems arise in the conduct of such trials. The first commented on by Chisholm, is the difficulty in assessing the value of prophylactic therapy in a population with little evidence

of folate deficiency the incidence of megaloblastic anaemia of pregnancy in her population being less than 1 per cent. For this reason very large numbers of patients may be required to obtain significant results.

The second difficulty arises from the fact that the daily dietary intake of folates is uncertain but may range from less than 50 μg to several hundred microgrammes Thus the nature of the diet may influence considerably the minimum supplementary requirements This probably accounts for the variation in results of trials in different areas.

The aim of prophylactic therapy should be to eliminate the occurrence of deficiency and the optimal dosage should be the minimum required to maintain a satisfactory folate balance in patients at known increased risk namely those with an insignificant dietary intake and with other factors such as high parity and previous anaemia especially if known to have been associated with folate depletion Allowance must also be made for the fact that in clinical practice as opposed to personally supervised trials, tablets are rarely taken with the recommended frequency

From available evidence it was concluded that a daily supplement of 500 μg folic acid should be satisfactory for the prevention of folate deficiency even in high risk patients and a triple blind trial was instituted to test this hypothesis

Clinical material

Mill Road Maternity Hospital Liverpool serves a population of predominantly poor social status low nutritional standards and high parity In a previous investigation the incidence of megaloblastic anaemia was shown to be approximately 5 per cent and biochemical evidence of defective folate metabolism, as judged by FIGLU excretion was demonstrated in 10–15 per cent of the hospital population (*Hibbard* 1964) Recurrence of defective metabolism was found in 73 per cent subsequent pregnancies (*Hibbard* and *Hibbard* 1966)

Only patients with a known history of defective folate metabolism in a previous pregnancy as judged by megaloblastic anaemia

excessive FIGLU excretion or low serum folate level (<2 ng/ml) were admitted to the trial. Selection of high risk patients in this way eliminates many of the problems found by other investigators in obtaining significant results.

No patient was admitted to the trial after 20 weeks gestation or if the haemoglobin level was 10.5 g per 100 ml or less. Patients were divided into three groups and received gelatine-coated capsules of identical appearance containing either 500 μg folic acid (Pregfol Wyeth) 5.0 mg folic acid or a placebo. In addition all capsules contained 60 mg elemental iron as ferrous sulphate. Dosage was one capsule daily. The patients were allocated consecutive numbers and the three capsules were issued by blind random selection at the source of issue. The code was not known whilst the trial was in progress. In view of the high risk of these women developing folate depletion, careful and detailed supervision by one or two clinicians was maintained as far as possible.

Laboratory investigations

Response to treatment was judged by assay of urinary formiminoglutamic acid (FIGLU) excretion and by serum folate levels at 4 week intervals. *Urinary FIGLU Excretion* was assessed following a 10 g histidine load. The method and assay using high voltage electrophoresis were as detailed by *Hibbard* (1964). Excretion of more than 20 μg/ml FIGLU in the urine passed 5 hours after histidine load was reported as a positive test. *Serum Folate Level* was measured on venous blood collected into folate-free sterile glass containers. Serum was stored at −20 °C without the addition of ascorbic acid. Assay was performed as soon as possible after collection of the specimen using a modification of *Herbert's* (1961) aseptic addition method. Using this technique we have found the normal range of values in non-pregnant subjects is 7–20 ng/ml. Values in pregnant patients fall to lower levels but for purposes of this investigation serum folate levels of less than 4.0 ng/ml were regarded as abnormal.

In addition *haemoglobin estimation* was performed on capillary blood using an EEL haemoglobinometer and an oxy-haemoglobin

Table I. *Results of Daily Prophylactic Administration to High Risk Patients of 60 mg Elemental Iron Alone and With 500 µg and 5 mg Folic Acid*

	No Folic Acid	500 µg Folic Acid	5 mg Folic Acid
Initial admissions to trial	26	27	26
Removed from trial after first attendance	8	10	5
Active participants with serial observations	18	17	21
Folate deficiency (FIGLU positive ± serum folate < 4 ng/ml) whilst on therapy	7	0	0
Secondary defaulters — non attendance or failure to take therapy	4	2	8
Folate deficiency subsequently shown in secondary defaulters	4	2	5

method. Values less than 10.5 g per 100 ml were regarded as indicative of anaemia.

Haemoglobin estimations were performed on every patient at each antenatal visit, this being the usual practice in the hospital.

Results

Seventy-nine patients were admitted to the trial but of these 23 were subsequently excluded, the principle reasons being default (12) and a positive FIGLU excretion test at the first visit (7). A further 14 patients defaulted subsequently but the results of treatment for at least 4 weeks were known in these patients.

The serial results of serum folate assays and FIGLU excretion tests are shown in Figures 1–3.

Control patients

In the control group of patients not receiving folic acid it is observed that the serum folate levels tend to fall as pregnancy progresses (Fig. 1), a trend noted by other investigators and this in spite of the fact that patients who developed excessive FIGLU

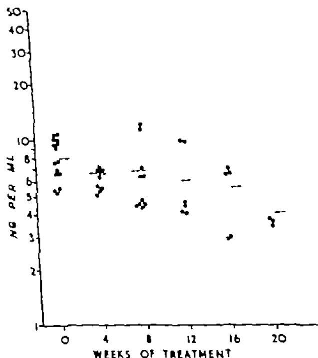


Fig. 1 Serial serum folate levels in patients with high risk of folate deficiency treated with 60 mg elemental iron daily. Patients developing folate deficiency were subsequently withdrawn from the trial and treated with folic acid

excretion were removed from the trial. The mean serum folate level at the time of admission to the trial was 8 0 ng/ml, but fell to 6 0 ng/ml 12 weeks later and after 20 weeks had fallen to 4 0 ng/ml.

Of the 26 patients initially admitted to the control group 5 were lost by primary default and 1 was given folic acid by her family doctor. Of the remaining 20 patients, 2 had positive

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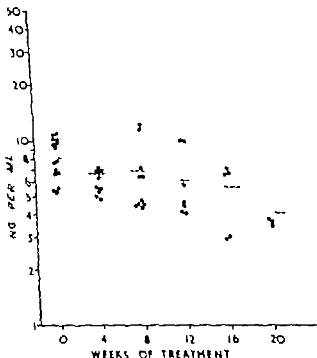


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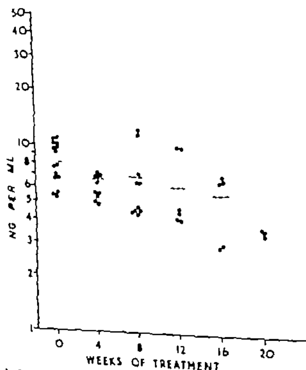


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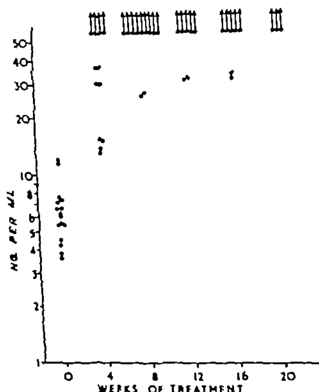


Fig. 3 Serial serum folate levels in patients with a high risk of folate deficiency treated with 5 mg folic acid and 60 mg elemental iron daily

Patients receiving folic acid

In both groups of patients receiving folic acid, serum folate levels rose within 4 weeks and generally were maintained at high levels thereafter (Figs. 2 and 3) Mean values could not be calculated, as many readings were above levels amenable to accurate assay But median values, after the initial rise, showed no significant subsequent variations, even with prolonged therapy

In the 500 μg dosage group the levels generally were above the

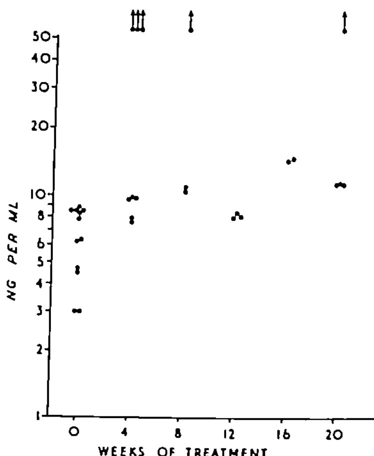


Fig — Serial serum folate leve s in patients with a high risk of folate deficiency treated with 500 μg fol c acid and 60 mg elemental iron daily

FIGLU excretion tests at their first visit and eleven developed positive tests subsequently Thus positive FIGLU excretion tests were found ultimately in 13 out of 20 patients available for serial study This incidence of 65 per cent is close to the expected incidence of 73 per cent found in a previous study of recurrent defective folate metabolism in the same population (*Hibbard* and *Hibbard* 1966) Eleven of the 18 active participants in the control series showed excessive FIGLU excretion as the trial progressed.

excretion tests associated in 2 instances with serum folate levels below 4 ng/ml Seven patients under active treatment with folic acid were anaemic but all had high serum folate levels and negative FIGLU excretion tests.

Apart from the patients with positive FIGLU tests, treatment was continued as before with the addition of a further 120 mg elemental iron daily as ferrous sulphate. In those cases available for adequate follow up thereafter a significant rise in haemoglobin level was shown.

Discussion

This study of groups of pregnant women, selected because of a high risk of defective folate metabolism, shows that 500 μg folic acid daily is adequate to prevent folate deficiency during pregnancy and is as effective as a dosage of 5 mg daily as judged by the incidence of excessive FIGLU excretion the serum folate levels and the haemoglobin concentration. The exceptionally high serum folate levels found in many patients receiving 5 mg folic acid daily to not appear to confer any clinical benefit.

It is evident from the figures in Table 1 that the population studied, as well as being nutritionally and economically inferior is characterized by a lax attitude towards personal health. In spite of close personal supervision it is notable that of the initial 79 patients 12 failed to attend the clinic again after the first visit and subsequently a further 14 defaulted or were known to have failed to take the prescribed therapy

It is therefore reasonable to assume that amongst the remaining patients there were many who did not take their capsules with the prescribed frequency Yet no case of folate depletion occurred amongst those patients receiving 500 μg folic acid daily suggesting that this dosage provides a reasonable safety margin even for the high risk poorly motivated patient.

Whilst the formulation suggested provides adequate protection from folate deficiency it may be questioned whether the iron content is adequate for a poorly nourished population, since some patients developed mild anaemia, presumably of iron deficient type Since the majority of these patients responded to treatment

Table II. *Serum Folate Levels Approximately 4 Weeks After Commencing Prophylactic Therapy*

	No Folic Acid	500 µg Folic Acid	5 mg Folic Acid
Patients investigated	18	17	21
Serum folate over 50 ng/ml	0	3	7
Serum folate over 10 ng/ml	2	7	20
Serum folate 4–10 ng/ml	14	8	1
Serum folate under 4 ng/ml	2	2	0

normal range but rarely reached the exceptionally high values found in the 5 mg dosage group Two patients receiving 500 µg daily had serum folate levels below 4 ng/ml 4 weeks or more after commencing therapy One of these results was probably erroneous as subsequently assay showed a level of 8.9 ng/ml The other patient showed repeatedly low levels with a maximum of 3 8 ng/ml although she denied failing to take the prescribed therapy

None of the patients who were given folic acid supplements had positive FIGLU excretion tests whilst taking therapy regularly However among the secondary defaulters both patients in the 500 µg group and 5 of the 8 in the 5 mg group were shown to be folate deficient when further opportunities for investigation occurred later in pregnancy

The mean *haemoglobin* levels in all groups showed consistent but small decreases from the initial readings.

Twenty weeks after commencing therapy the mean decrease in haemoglobin level was as follows

No folic acid	0 75 G/100 ml
500 µg daily	1 5 G/100 ml
5 mg daily	1 6 G/100 ml

These differences are not statistically significant.

There was no case of severe anaemia the lowest haemoglobin level recorded being 9 6 G/100 ml However a haemoglobin concentration consistently below 10 5 G/100 ml was found in 7 patients in the control group and 4 of these had positive FIGLU

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with additional iron it is concluded that more satisfactory prophylaxis of anaemia would be achieved by a formulation offering a higher dosage of elemental iron providing that this does not result in an increase in adverse gastro-intestinal symptoms.

SUMMARY

Even in bad risk populations a regime offering a single daily capsule comprising 500 μg folic acid and 60 mg elemental iron gives effective protection from folate deficiency but may not be invariably effective in preventing iron deficiency However if satisfactory prophylaxis of anaemia as well as folate deficiency is to be achieved a higher iron content may be considered desirable.

Acknowledgements

We are indebted to the Medical Research Council and the Research Committee of the United Liverpool Hospitals for grants supporting the laboratory investigations reported here

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THE TREATMENT OF FOLATE DEFICIENCY IN PREGNANCY

BY

BRYAN M. HIBBARD AND ELIZABETH D. HIBBARD

The efficacy of daily supplements of folic acid in the microgramme range for the prophylaxis of folate deficiency in pregnancy is now well accepted but lower dosage regimes have not been so widely adopted for the treatment of established folate deficiency

The purpose of the trial described here was to assess the value of oral treatment of folate deficiency with 500 µg folic acid three times daily as compared with the traditional dosage of 5 mg three times daily

Clinical material

All the patients admitted to the trial were booked for confinement at Mill Road Maternity Hospital Liverpool and their gestation period was less than 36 weeks. The primary criterion for admission was the demonstration of excessive urinary FIGLU excretion, assayed by high voltage electrophoresis (Hibbard 1964) This was in most cases associated with anaemia and/or low serum folate levels or an obstetric history suggestive of defective folate metabolism The investigation was conducted as a double blind trial and patients were allotted to treatment groups randomly Two types of capsule of identical appearance were used Both contained 60 mg elemental iron as ferrous sulphate but the folic acid content was either 500 / g or 5 mg. The capsules were prescribed to be taken three times daily so that patients all received 180 mg elemental iron and either 1.5 mg or 15 mg folic acid daily

Table 1 *Results of Treatment of Defective Folate Metabolism with 180 mg Elemental Iron Plus 1.5 mg or 15 mg Folic Acid Daily*

	1.5 mg Folic Acid + 180 mg Iron Daily	15 mg Folic Acid + 180 mg Iron Daily
Initial admissions to trial	97	100
Subsequently excluded from trial	33	26
Active participants with serial observations	64	74
FIGLU test positive after 4 weeks therapy or became positive again later in pregnancy	5 (8%)	6 (8%)
Low folate level initially (< 4 ng/ml)	33	43
Low folate after 4 weeks therapy	0/27 investigated	1/26 investigated
Low haemoglobin level initially (10.5 G/100 ml)	28	40
Low Hb after 4 weeks therapy	8	19
Low Hb after 8 weeks therapy	3/11 investigated	5/23 investigated

Laboratory investigations

Serum folate levels and urinary FIGLU excretion were determined at 4 weekly intervals as detailed previously (*Hibbard* and *Hibbard* 1969) Haemoglobin concentration was estimated at every antenatal visit.

Results

One hundred and ninety seven patients were admitted to the trial but of these 59 were subsequently excluded principally because of default (32) or because folic acid had been prescribed from other sources (19)

FIGLU excretion

The FIGLU excretion test remained positive or again become positive later in pregnancy in 5 (8 per cent) of the low dosage group and 6 (8 per cent) of the high dosage group In the former group all the serum folate levels were normal but 3 patients had

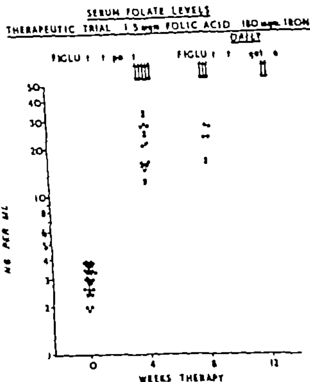


Fig. 1. Serial serum folate levels in patients with defective folate metabolism (initial serum folate level less than 4 ng/ml and excessive FIGLU excretion) treated with 1.5 mg folic acid and 180 mg elemental iron daily

haemoglobin concentrations under 10.5 G/100 ml. In the latter group 1 patient had a low serum folate (1.9 ng/ml) and 2 were anaemic

Serum folate levels

The serum folate levels in both groups generally rose within 4 weeks and were maintained at normal or high levels. Because of limitations imposed by the assay method accurate readings above 50 ng/ml could not be obtained and therefore mean values could not be determined, but it is evident that after 4 weeks therapy

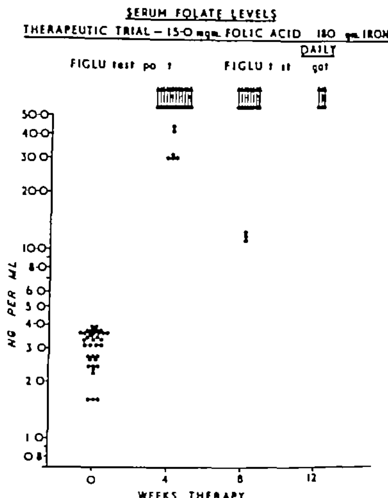


Fig. 2. Serial serum folate levels in patients with defective folate metabolism (Initial serum folate level less than 4 ng/ml and excessive FIGLU excretion) treated with 15 mg folic acid 180 mg elemental iron daily

almost all readings were within or above the normal range and very high levels were more commonly seen in the high dosage group

Low serum folate levels (under 4 ng/ml) in addition to excessive FIGLU excretion were found at the initial examination in 33 (52 per cent) of the low dosage group and 43 (58 per cent) of the high dosage group After 4 weeks therapy none of 27 patients investigated in the low dosage group and one of the 26 investigated in the high dosage group still had a low serum

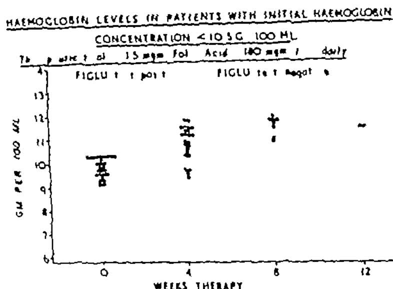


Fig 3 Serial haemoglobin estimations in patients with initial haemoglobin concentrations under 10 5 G/100 ml treated with 1.5 mg folic acid and 180 mg elemental iron daily

folate level (Table I) The solitary failure (folate 1.9 ng/ml) also had a positive FIGLU test and was anaemic It seemed likely that she had not taken the prescribed therapy as a further folate assay gave a result of 2 3 ng/ml

Haemoglobin concentration

After 4 weeks of treatment both groups showed a statistically significant (at the 1 per cent level) rise in haemoglobin concentration There was no significant difference between the responses obtained with low dosage and high dosage of folic acid, but there seemed to be greater variability in the results with the lower dosage

The response of those patients with a low initial haemoglobin concentration (under 10 5 G-100 ml) was considered separately In the low dosage group there were 28 such patients and 15 of these had a serum folate level below 4 ng/ml. After 4 weeks of

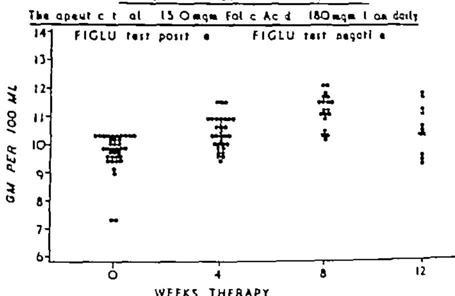


Fig. 4 Serial haemoglobin estimations in patients with initial haemoglobin concentrations under 10.5 G/100 ml treated with 15 mg folic acid and 180 mg elemental iron daily

therapy 8 of the 28 patients still had low haemoglobin levels al though their folate levels and FIGLU excretion were normal. In the high dosage group 40 patients had an initial haemoglobin level below 10 5 G/100 ml and 24 of these had a serum folate level below 4 ng/ml After 4 weeks therapy nineteen patients remained anaemic although only one of these had a positive FIGLU ex cretion test associated with a serum folate level of 1.9 ng/ml.

Discussion

The most important observation in this comparative trial is that there was no significant difference in the efficacy of the low dosage and high dosage regimes as assessed by any of the para meters studied that is reversion of FIGLU excretion to normal, rise in serum folate level to above 4 ng/ml and correction of anaemia

As judged biochemically the response was generally satisfac

tory but the persistance or recurrence of excessive FIGLU ex cretion in 8 per cent patients in both groups suggests that some patients have an intrinsic metabolic defect in addition to or rather than, a deficient intake. This is supported by the fact that with one exception this group of patients had serum folate levels within or above the normal range at the time of their subsequent positive FIGLU excretion tests. Also 3 out of 5 patients in the low dosage group and 2 out of 6 in the high dosage had continued clinical evidence of anaemia. It is notable that this figure of 8 per cent failure to respond to high dosage oral folic acid therapy is *exactly the same* as that reported in a previous study in a large series of patients from the same hospital (*Hibbard* 1967) This emphasizes the need for continued clinical and laboratory supervision of patients for whom folic acid therapy has been prescribed.

The importance of factors other than dietary inadequacy of iron and folic acid, and the need for regular supervision is also shown by the poor haemoglobin response of some patients. The reasons for this are often easily determined, for example the presence of helminthic infestations, chronic insidious blood loss, or chronic urinary infection but sometimes less obvious factors such as inborn metabolic errors associated with defective haemoglobin synthesis operate

Even the low dosage regime used in this trial may be unnecessarily large for effective therapy The choice of dosage was based principally on theoretical considerations and the results of trials w th microgramme dosage for prophylaxis. If 500 μg daily was adequate for prophylaxis it was considered that three times this amount would provide sufficient folic acid for normal daily requirements, for the additional demands which would accompany the restoration of normal cellular activity and increased haemopoiesis and would enable depleted reserves to be replenished.

In addition, t is convenient to use the same formulation of paule as is used for prophylaxis. The increased daily dose provides 180 mg elemental iron which should be adequate for the treatment of concomitant iron deficiency which is commonly seen in ssociation with folate deficiency

Further trials using even lower doses of folic acid would be of

interest to determine the theoretical needs for folic acid in depleted patients, but for widespread clinical application it must be born in mind that a reasonable safety margin is required and allowance must be made for variations in individual requirements, absorption and failure to take prescribed treatment. In the present study there was a suggestion of greater fluctuations in haemoglobin response amongst patients in the low dosage group and this could imply that the dosage was close to the requirements in some cases.

SUMMARY

A trial comparing the value of daily doses of 1 5 mg and 15 mg folic acid given orally for the treatment of folate deficiency in pregnancy shows no significant differences between the two groups as judged by excessive FIGLU excretion low serum folate levels and haemoglobin concentrations

It is concluded that 1.5 mg folic acid daily is adequate for the treatment of folate deficiency in pregnancy The importance of continued clinical and laboratory supervision of patients receiving treatment is emphasised since a small proportion of patients fail to show a satisfactory response to therapy even taking 15 mg folic acid daily

Acknowledgements

We are indebted to the Medical Research Council and the Research Committee of the United Liverpool Hospitals for grants supporting the laboratory investigations reported here

We are also grateful to Dr D Richards and Messrs. John Wyeth and Co Ltd for the preparation and supply of the haematinic capsules

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GASTROINTESTINAL PROTEIN LOSS IN TOXAEMIC PATIENTS

BY

L. LAAKSO AND J. PAASIO

Semi-quantitative radioactive isotope studies have given significant additional diagnostic help in the analysis of intestinal plasma protein loss. The most common techniques involve the use of Iodine-131 labelled albumin (RIHSA 131) (*Jarnum* 1961 a) or Polyvinylpyrrolidone (PVP I 131) (*Gordon* 1959) and the measurement of radioactive I 131 in the faeces. Intestinal plasma protein loss is a non-specific finding associated with several diseases of the gastrointestinal tract. For example massive protein loss in the faeces may be caused by hypertrophic gastritis, gastric carcinoma, the sprue syndrome, ulcerative colitis, salmonellosis, lymphomas, obstruction of the thoracic duct, essential intestinal lymphangiectasia and a fall in venous pressure in the systemic circulation (*Schwarz et al.* 1967). Plasma protein loss is also caused by certain general conditions accompanied by disturbances in capillary permeability such as shock, radiation injuries and nephrosis (*Barandun et al.* 1965). In view of these facts we decided to study the possible plasma protein loss in the faeces of toxaemic patients.

Material and Method

Sixteen parturients were studied. Six had normal pregnancies, four had pyelitis of pregnancy and six had preeclampsia with toxaemia. A total of 44 faecal determinations were made. Each subject was given immediately *post partum* 15 drops of potas-

interest to determine the theoretical needs for folic acid in depleted patients but for widespread clinical application it must be born in mind that a reasonable safety margin is required and allowance must be made for variations in individual requirements, absorption and failure to take prescribed treatment. In the present study there was a suggestion of greater fluctuations in haemoglobin response amongst patients in the low dosage group and this could imply that the dosage was close to the requirements in some cases.

SUMMARY

A trial comparing the value of daily doses of 1.5 mg and 15 mg folic acid given orally for the treatment of folate deficiency in pregnancy shows no significant differences between the two groups as judged by excessive FIGLU excretion low serum folate levels and haemoglobin concentrations.

It is concluded that 1.5 mg folic acid daily is adequate for the *treatment of folate deficiency in pregnancy* The importance of continued clinical and laboratory supervision of patients receiving treatment is emphasised since a small proportion of patients fail to show a satisfactory response to therapy even taking 15 mg folic acid daily

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Material and Method

Sixteen parturients were studied. Six had normal pregnancies four had pyelitis of pregnancy and six had preeclampsia with toxaemia. A total of 44 faecal determinations were made. Each subject was given immediately post partum 15 drops of potas-

sium iodide three times a day for three days. She was then given 10 μCi of PVP I 131 by intravenous injection. Collection of faeces began after six hours or from the first subsequent defaecation and was continued for 48 hours. The specimens for each day were collected separately. Each specimen was homogenised and its radioactivity was measured and expressed as a percentage of the dose administered. Preliminary studies revealed that the maximum PVP I 131 excretion occurs within the period of 48 hours. The collection time varied from two to eight days (e.g. *Gordon* 1959, *Jarnum* 1961 a). In the cases of toxaemia, a further specimen was collected 6–8 weeks *post partum*. Urinary protein excretion, urinary sedimentation and serum protein were also studied in relation to both periods of faecal collection.

Results

The results are presented in its entirety in Table I. The excretion of PVP I 131 in the faeces of the six normal parturients (12 samples) immediately *post partum* was 0.121 ± 0.058 per cent/24 hours. In the four parturients who had urinary tract infection with proteinuria (eight samples) it was 0.088 ± 0.042 per cent/24 hours. There is no significant difference between these two groups. However, the mean PVP I 131 excretion in the 24-hour faecal specimens (12 samples) collected from the six toxaemic patients immediately *post partum* was 0.490 ± 0.503 and after 6–8 weeks it was 0.322 ± 0.240 per cent/24 hours. The difference between the PVP I 131 excretion values of normal and toxaemic parturients immediately *post partum* is significant ($0.01 \leq p \leq 0.05$). The difference is less marked ($0.125 \leq p \leq 0.250$) when comparison is made between the faeces collected of 6–8 weeks from the patients with toxaemia. No distinct correlation with proteinuria is demonstrable. This emerges e.g. in Cases 14 and 16 where the excretion of proteinuria per 24 hours is the same but the corresponding PVP I 131 excretion in the faeces differs markedly. The serum protein values for toxaemic patients rose slightly 6–8 weeks after delivery. No hypoproteinemia was evident in any of the cases.

Table 1 Result Immediately Post Partum (All Patients) and 6–8 Weeks after Delivery (Toxaemic Patients)

Cases	Parity	Diagnosis	Results Immediately after Delivery: Bacteriuria	Serum-protein (%)	Proteinuria g/24 h: 1	Proteinuria g/24 h: 2	PVP-I-131 in Faeces (%): 1	PVP-I-131 in Faeces (%): 2	6–8 Weeks after Delivery: Bacteriuria	Serum-protein (%)	Proteinuria g/24 h		PVP-I 131 in Faeces (%)	
1	I	Normal pregnancy	0	6.1	0	0	0.04	0.06						
2	I	Normal pregnancy	0	6.2	0	0	0.12	0.18						
3	I	Normal pregnancy	0	6.5	0	0	0.14	0.19						
4	I	Normal pregnancy	0	7.3	0	0	0.09	0.24						
5	IV	Normal pregnancy	0	7.1	0	0	0.15	0.06						
6	I	Normal pregnancy	0	7.0	0.1	0	0.15	0.04						
7	II	Pyelitis of pregnancy		6.0	0	0	0.01	0.10						
8	I	Pyelitis of pregnancy	+	5.8	1.0	1.0	0.13	0.16						
9	I	Pyelitis of pregnancy		6.0	1.0	0	0.02	0.10						
10	I	Pyelitis of pregnancy		6.0	0.9	0.1	0.11	0.08						
11	I	Pre-eclampsia gravis	0	6.3	4.0	2.4	0.34	0.42	0	6.8	0	0	0.41	0.45
12	II	Pre-eclampsia levis	0	6.5	1.0	1.0	0.43	0.23	0	6.9	0	0	0.23	0.27
13	I	Pre-eclampsia gravis	0	6.4	2.7	2.3	0.27	0.30	0	7.5	0.1	0.1	0.27	0.35
14	I	Pre-eclampsia levis	0	6.3	0.1	0.1	0.88	1.98	0	7.3	0	0	1.00	0.15
15	I	Pre-eclampsia gravis	0	7.2	2.1	2.5	0.16	0.59	0	7.0	0.1	0	0.04	0.29
16	I	Pre-eclampsia levis	0	6.0	0.1	0.1	0.18	0.10	0	7.2	0	0	0.15	0.11

Abbreviations 1 = The first day 2 = The second day

sium iodide three times a day for three days. She was then given 10 μCi of PVP I 131 by intravenous injection. Collection of faeces began after six hours or from the first subsequent defaecation and was continued for 48 hours The specimens for each day were collected separately Each specimen was homogenised and its radioactivity was measured and expressed as a percentage of the dose administered. *Preliminary studies* revealed that the maximum PVP I 131 excretion occurs within the period of 48 hours The collection time varied from two to eight days (*e g* *Gordon* 1959 *Jarnum* 1961 a) In the cases of toxaemia, a further specimen was collected 6–8 weeks *post partum* Urinary protein excretion urinary sedimentation and serum protein were also studied in relation to both periods of faecal collection.

Results

The results are presented in its entirety in Table I The excretion of PVP I 131 in the faeces of the six normal parturients (12 samples) immediately *post partum* was 0 121 ± 0 058 per cent/24 hours. In the four parturients who had urinary tract infection with proteinuria (eight samples) it was 0 088 ± 0 042 per cent/24 hours. There is no significant difference between these two groups However the mean PVP I 131 excretion in the 24-hour faecal specimens (12 samples) collected from the six toxaemic patients immediately *post partum* was 0 490 ± 0 503 and after 6–8 weeks it was 0 322 ± 0 240 per cent/24 hours. The difference between the PVP I 131 excretion values of normal and toxaemic parturients immediately *post partum* is significant (0 01 ≤ p ≤ ≤ 0 05) The difference is less marked (*0 125 ≤ p ≤ 0 250*) when comparison is made between the faeces collected of 6–8 weeks from the patients with toxaemia No distinct correlation with proteinuria is demonstrable This emerges *e g* in Cases 14 and 16 where the excretion of proteinuria per 24 hours is the same but the corresponding PVP I 131 excretion in the faeces differs markedly The serum protein values for toxaemic patients rose slightly 6–8 weeks after delivery No hypoproteinemia was evident in any of the cases

SUMMARY

The PVP-I 131 faecal excretion test was performed on parturients immediately *post partum*. Faeces were collected separately for 48 hours. For twelve 24-hour samples collected from six normal parturients the PVP I 131 excretion value was 0.121 ± 0.058 per cent. It was *0.088 ± 0.042* per cent/24 hours for four patients with pyelitis of pregnancy (eight samples). The value for six patients with toxaemia (12 samples) was 0.490 ± 0.503 per cent/24 hours, a statistically significant difference from the normal parturient value. Excretion decreased to 0.322 ± 0.240 per cent after 6–8 weeks. The finding must be regarded as semiquantitative. The increase in plasma protein loss in the cases of toxaemia is probably due to alterations in permeability.

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Discussion

The considerable protein loss that may occur through the gastro-intestinal tract is associated with the active synthesis of digestive enzymes and the rapid catabolism of epithelial tissue. Plasma proteins occur normally in both gastric and intestinal juice and are digested like other proteins during the normal digestive processes. The catabolism of albumin occurs most rapidly in the duodenum (*Wetterfors* 1965)

The mucosa of the *gastrointestinal tract* lacks the screening test" possessed by *e g* the renal glomeruli The permeability of these glomeruli to protein fractions is dependent on the weight of the protein molecule even in an abnormal state (*Kekki* 1967) The molecular weight of PVP I 131 is c. 40 000 and it behaves like plasma protein unless the I 131 bond breaks. This is most likely to occur if the PVP preparation is old (*Löbe et al.* 1966) According to *Jarnum* (1961 b) faecal excretion of PVP in normal cases is 0–0 25 per cent/24 hours while in pathological cases it can even be 5 per cent/24 hours. The values for the toxaemic patients immediately *post partum* exceeded these normal values, whereas the results for the control and pyelitis still had higher excretion values than the controls after 6–8 weeks, but they were lower than the values immediately *post partum*.

The factors influencing albumin synthesis and catabolism are not precisely known. The maximum synthesising capacity of the liver is roughly three times the accepted normal (*Koblet* 1965) In contrast the body is capable of increasing many times the synthesis of gamma globulin Hypogammaglobulinemia caused by accelerated catabolism is associated with *e g* the nephrotic syndrome which is also manifest clinically as protein loss via the kidneys and the intestines This may be connected with changes in capillary permeability (*Waldmann* and *Laster* 1964) Corresponding changes in capillary permeability have been established in parturients (*Friedberg* and *Lutz* 1963) These are not, however correlated with the degree of severity of toxaemia (*Castrén* and *Laakso* 1965) or was any correlation established in the present work with the 24-hour excretion of proteinuria. This reduction in capillary permeability in patients with toxaemia may also be the reason for the protein losing enteropathy

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in the other because of foetal distress. The babies were all healthy and mature.

During labour the patients were all given nitrous oxide. Trichlorethylene or in a few cases chloroform was also administered at the end of the second stage of labour

A non-pregnant control group consisted of fourteen healthy volunteers. The age distribution in the non-pregnant and the pregnant groups was similar

Methods

1 *General procedure*

In all subjects the carbon monoxide (CO) production, carboxy haemoglobin (COHb) saturation, haemoglobin (Hb) concentration and *total amount of haemoglobin* (THb) were measured.

In 20 of the pregnant women the measurements were made before and 0–24 hours after delivery and in 10 of these the external blood loss was measured as described by *Robbe* and *Ström* (1958) *except that the blood was collected only for up to two* hours post-partum. In the 13 remaining women no measurements were made after delivery due to Caesarian section excessive bleeding, lack of cooperation etc.

CO-production was measured according to *Linderholm* (1968)

Total haemoglobin in the body (THb) was measured in conjunction with the measurement of CO production, either directly afterwards, or if the rebreathing procedure was tiring for the subject a few hours later Most of the pregnant women preferred to be on their sides instead of in the usual semirecumbent position

COHb saturation in blood was measured according to the alveolar CO method (*Sjöst and* 1948) A closed rebreathing system with O_2 and CO_2 absorption was used and the rebreathed gas was analysed.

For the CO analysis in gas three methods were used.

1 *The palladium-molybdenum tube method* (*Andersson* and *Dahlström*, 1958 and *Dahlström*, 1960)

2 *The hopcalite method* (*Linderholm* and *Sjöstrand* 1956)

Linderholm, H and Lundström, P Acta obstet et gynec scandinav 48, 362, 1969

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ENDOGENOUS CARBON MONOXIDE PRODUCTION AND BLOOD LOSS AT DELIVERY

BY

H. LINDERHOLM AND P LUNDSTRÖM

Robbe Ström and *Zetterström* (1960) who found a considerable increase in the percentage saturation of carboxyhaemoglobin in the blood two hours after delivery suggested that increased haemolysis might at least partially explain the "hidden blood loss found to occur at delivery (*Löwenstein Pick* and *Philpott* 1950 *Verel Bury* and *Hope* 1956 and *Robbe* and *Ström* 1958) If increased haemolysis occurs this may be expected to give an increased production of carbon monoxide which it is now possible to measure (*Coburn Blakemore* and *Forster* 1963 *Linderholm* 1968) In order to investigate these problems the carbon monoxide production before and after delivery was studied

Material

Thirty three healthy pregnant women all non-smokers from a maternal welfare organization were included in the study The pregnancy of these women was uneventful. For instance none had symptoms of toxaemia and none were Rh-iso-immunized. Three subjects had a moderate sideropenic anaemia.

Where post partum measurements were performed the delivery took place without or with only minor complications. No blood transfusion was given. In some cases episiotomy was carried out. In two cases vacuum extraction was performed, in one case owing to a slight prolongation of the second stage of labour and

Table I. Comparison Between the Values of the COHb Saturation in Per Cent Obtained by Different Methods of Analysing the CO Content of the Rebreathed Gas

Period of Examination		Palladium-Molybdenum Tube Method	Hopcalite Method	Infra-red Method
Ante partum	n	11	11	11
(1 10 hrs)	M	0.44	1.20	6 61
	Md	0.45	0 47	0.61
	R	0 76 – 0.61	0.35 6.60	0 48 – >0.30
	S D	0.465	1.25	6.99
Post partum		10	10	10
(0–2 hrs)	M	0.64	1.02	10.39
	Md	0.60	0.71	8.55
	R	0.38 1.10	0 40 – 2.57	2.2 – 23.8
	S D	0.676	1.07	10.9
Post partum	n	9	9	9
(2–8 hrs)	M	0.67	0 62	5 00
	Md	0.60	0.53	3.10
	R	0.38 1 15	0.40 – 1.05	0.81 9.60
	S D	0.715	0.654	5.30
Post partum		11	11	11
(8–24 hrs)	M	0 61	0 58	1 08
	Md	0 57	0.58	0.82
	R	0 46 0 84	0 42 0.72	0.54 3.90
	S D	0 639	0 607	1 13

All the women, some of whom were smokers, were given N_2O. During the last period of the second stage of labour the N_2O was supplemented with $CHCl_3$ in 5 cases, and with $CHCl\,CCl_2$ in 2 cases. The dose and the duration of administration varied widely. Some subjects had received N_2O before the antepartum measurements were carried out.

During this period only N_2O was given.

Symbols: n number of cases, M mean, Md median, R range, S D standard deviation

The postpartum values obtained with the hopcalite meter are similar to those found by *Robbe, Ström* and *Zetterström* (1960).

The sensitivity of the infra red and the hopcalite meters to N_2O is the main cause for the discrepancy between the results

using a Stålex CO meter and a Philips potentiometer writer (OR 2210 A 21)

3 *The infra red absorption method* A Beckman LIB infrared analyser model 15 A was used in combination with a Philips potentiometer writer Dry gas was obtained by using a $CaCl_2$ filter

All methods were continuously checked by calibration with standard CO gas mixtures.

2. *The specificity of the methods for CO analysis*

None of the three methods used for analysis of CO in gas are entirely specific for CO Anaesthetic agents in particular may cause *interference* As the *patients had inhaled nitrous oxide* (N_2O) and in some cases trichlorethylene ($CHCl\,CCl_2$) or chloroform ($CHCl_3$) the sensitivity of the analytical methods to these gases was examined.

It was found that the palladium molybdenum test tube method *was quite insensitive to* N_2O $CHCl_3$ *and* $CHCl\,CCl_2$ even in high concentrations

The hopcalite meter was 50–100 times more sensitive to CO than to $CHCl_3$ and $CHCl\,CCl_2$ and about 2000 times more sensitive to CO than to N_2O (concentrations in mols/litre)

When mixtures of N_2O *and* $CHCl_3$ *were analysed* the deflection of the CO meter was less than the sum of the deflections caused by each gas separately

The infra red meter was about 10 times more sensitive to CO than to N_2O $CHCl_3$ and $CHCl\,CCl_2$ did not produce any deflection when this meter was used.

The concentration of the anaesthetic gases may be expected to be high in relation to that of CO in expired air In order to study the effect on the analytical methods of the anaesthetics used 11 pregnant *women were examined under conditions similar* to those of the present study

Table I shows the erroneously high COHb saturation obtained when the results are based on CO analysis of the rebreathed gas with the hopcalite or the infra-red meter

Table I. *Comparison Between the Values of the COHb Saturation in Per Cent Obtained by Different Methods of Analysing the CO Content of the Re-breathed Gas*

Period of Examination		Palladium-Molybdenum Tube Method	Hopcalite Method	Infra-red Method
Ant partum		11	11	11
(1 10 hrs)	M	0.44	1.20	6.61
	Md	0.45	0.47	0.61
	R	0.26 – 0.61	0.35 – 6.60	0.48 – >0.30
	S.D.	0.465	1.25	6.99
Post partum	n	10	10	10
(0–2 hr.)	M	0.64	1.02	10.39
	Md	0.60	0.71	8.55
	R	0.38 1.10	0.40 – 2.57	2.2 – 23.8
	S.D	0.676	1.07	10.9
Post partum	n	9	9	9
(2–8 hrs)	M	0.67	0.62	5.00
	Md	0.60	0.53	3.10
	R	0.38 1.15	0.40 – 1.05	0.81 9.60
	S.D	0.715	0.654	5.30
Post partum		11	11	11
(8–24 hrs)	M	0.61	0.58	1.08
	Md	0.57	0.58	0.82
	R	0.46 0.84	0.42 0.72	0.54 3.90
	S.D	0.639	0.607	1.13

All the women some of whom were smokers, were given N_2O. During the last period of the second stage of labour the N_2O was supplemented with $CHCl_3$ in 5 cases, and with $CHCl\,CCl_2$ in 2 cases. The dose and the duration of administration varied widely. Some subjects had received N_2O before the antepartum measurements were carried out.

During this period only N_2O was given.

Symbols: n number of cases, M mean, Md median, R range, S.D. standard deviation.

The postpartum values obtained with the hopcalite meter are similar to those found by *Robbe, Ström* and *Zetterström* (1960).

The sensitivity of the infra-red and the hopcalite meters to N_2O is the main cause for the discrepancy between the results

obtained with the palladium-molybdenum method on the one hand and the hopcalite and infra red methods on the other

It was found that even before N_2O was administered, the presence of N_2O in the air of the delivery room was sufficient to affect the results of the analysis with the hopcalite and infra-red meters.

In addition $CHCl_3$, and probably even $CHCl\ CCl_2$, contributed to the erroneously high COHb concentration obtained with the hopcalite method in the period 0–2 hours after delivery

In some cases the COHb saturation of the blood was directly determined with the palladium-molybdenum tube micromethod (*Linderholm* 1965) and with the method of *Linderholm Sjöstrand* and *Söderström* (1966) The samples were taken before and after delivery at the end of the rebreathing procedure. The results which are not accounted for in the tables agreed well with those obtained with the alveolar method using the palladium-molybdenum tube method for CO analysis but not with those obtained with the hopcalite and infra red methods.

Unless filters are used the hopcalite method is unsuitable for CO analysis if N_2O or $CHCl_3$ (and probably $CHCl\ CCl_2$) is present in the respiratory gas The same is true for the infra red meter as far as N_2O is concerned Therefore all figures under Results are based on CO analysis with the palladium-molybdenum test tube method.

Results

According to Table II neither the *Hb concentration* nor the *COHb saturation* changed appreciably up to 24 hours after delivery The mean CO *production* seemed to be almost the same before and after delivery even when the calculations were based on the individual differences The mean CO production was somewhat lower in the control group than in the group of pregnant women while the rate of CO production per g THb was approximately the same in both groups. Calculated from the individual differences the mean CO production per hour per g THb before delivery and up to 24 hours after delivery showed rather small and statistically insignificant changes

Table II Haemoglobin Concentration Total Amount of Haemoglobin (THb) Saturation of Hb with CO (COHb) Total CO Production, and CO Production per g THb in Pregnant Women before and 0–24 Hours after Delivery and in a Control Group of Non-pregnant Women

		n	Hb Conc. g/100 ml	THb g	COHb per cent	CO Production ml/hr	CO Production µl/hr/g THb
			Mean and standard deviation				
Non-pregnant controls		14	10.7 ± 0.85	460 ± 76	0.44 ± 0.11	0.39 ± 0.23	0.90 ± 0.51
Pregnant women							
Ante partum		33	10.5 ± 2.3	622 ± 119	0.40 ± 0.11	0.59 ± 0.38	0.98 ± 0.61
0–2 hrs pp		9	11.6 ± 1.2	564 ± 117	0.36 ± 0.09	0.58 ± 0.20	1.06 ± 0.44
0–8 h pp		13	11.5 ± 1.0	566 ± 113	0.35 ± 0.09	0.60 ± 0.19	1.10 ± 0.39
8–24 hrs pp		14	11.1 ± 1.2	541 ± 106	0.38 ± 0.11	0.67 ± 0.32	1.22 ± 0.43
			Mean and standard deviation of the individual differences				
0– hr pp	p	9	0.32 ± 0.96	47 ± 92	0.04 ± 0.11	−0.13 ± 0.47	0.06 ± 0.71
0– hr pp	p	13	0.16 ± 0.83	70 ± 101	−0.07 ± 0.14	0.08 ± 0.49	+0.07 ± 0.72
–4 hr pp	p	14	0.01 ± 1.20	71 ± 73	0.06 ± 0.14	+0.09 ± 0.51	0.23 ± 0.86
0–4 hrs pp	p	20	0.30 ± 0.99	66 ± 87	−0.04 ± 0.13	±0.00 ± 0.49	0.11 ± 0.76

p p post partum   p ante partum

Table III *Total Amount of Haemoglobin (THb) ante and post partum, and External Blood Loss at Delivery* (10)

	Mean	Range
THb g, 2 10 days ante partum	641	495–770
THb g, 3–5 days post partum	534	450–622
Total Hb loss (difference THb ante–post partum)	108	10–175
External Hb loss g, up to 2 hours post partum	30	15–56
Difference between "total" and "estimated external" Hb loss g[1]	42*	35–106

The "estimated external" blood loss was calculated in the following way To the mean loss of 30 g of Hb up til 2 hours post partum 7 g (2 hours post partum–4 days post partum) 14 g (placental loss) and 13 g (=Foetal loss) were added according to Rabbe and Sirdén (1958)

P 0.01

The *total amount of haemoglobin* (THb) in the 10 subjects in whom the external blood loss at delivery was measured was on the average 106 g less 3–5 days after delivery than before delivery At delivery and during the first 4 *post partum* days the estimated average external blood loss was 64 6 g Hb The difference is 42.2 ± 14 6 g Hb ($P < 0\ 01$)

Discussion

CO *production*

The COHb saturation reflects the rate of CO production but it is also influenced by such factors as the CO concentration in ambient air and the alveolar ventilation (*Coburn et al.* 1963) During pregnancy and labour hyperventilation and a high O_2 consumption is present (*Cugell Frank* and *Gaensler* 1953 *Cohen* and *Thomson* 1939) After delivery O_2 consumption and ventilation decrease Therefore the rate of CO production is best measured directly

In our series the mean CO production of the pregnant women, 0 59 ml CO per hour was somewhat higher than that of the healthy non pregnant controls 0.39 ml CO per hour *Coburn et al.* (1963) using a somewhat different method found the mean production in a group of 10 young men to be 0 42 ml CO per hour

The CO produced by the body originates mainly from the breakdown of Hb (*Coburn et al.* 1964 *Luomanmäki* 1966) It was therefore of interest to study the rate of CO production per g THb As this was approximately equal in pregnant women during the last month of pregnancy and in non pregnant women it seems likely that the increase in THb during pregnancy accounts for most of the increase of the CO production rate The CO production per g THb is higher in our groups than that reported by *Coburn Forster* and *Kane* (1965) The difference is probably due to methodological dissimilarities.

After delivery the rate of CO production did not change significantly in spite of the decrease in THb This may indicate a relatively high rate of CO production during the first 24 hours after delivery that may be related to lesions of the parturient canal and to degeneration of the decidual tissue An increase of

COHb concentration indicating increased haemolysis after surgery has been described (*Ibring*, 1958/59)

COHb saturation

According to Table II the COHb saturation before and after delivery was unchanged, but in Table I an increase after delivery is found. This statistically insignificant increase was due to a high COHb concentration in two subjects who were smokers. Our results differ from those obtained by *Robbe et al* (1960) who found a 240 per cent increase in the COHb saturation two hours after delivery This may be due to an analytical error as the hopcalite apparatus they used is sensitive to anaesthetic gases (see under Methods")

The loss of haemoglobin at and after delivery In our investigation the THb was considerably lower 3–5 days after delivery than before delivery This decrease in THb exceeds the estimated xternal loss of Hb at and after delivery (see footnote Table III) This is in agreement with the findings of *Löwenstein et al* (1950) *Verel et al* (1956) and *Robbe* and *Ström* (1958)

Assuming that the haemolysis of 1 g of Hb results in the formation of 1 34 ml of CO 40–55 ml CO will be produced by the haemolysis of 30–40 g Hb. As a "hidden loss of this magnitude seems to take place at or immediately after delivery an increased COHb saturation would without doubt be found if the loss in question depended to any great extent on haemolysis. We found no such increase In addition, the change in the rate of CO production was negligible The hypothesis that the "hidden blood loss after delivery is partly due to haemolysis is not sustained by our investigation For the time being we can offer no explanation of the fall in THb at deli ery and during the first four postpartum days.

SUMMARY

The ate of CO production was determined in 33 pregnant women during the last month of pregnancy and during the first 24 hours after deli ery and in 14 healthy non pregnant controls. It was approximately the same in the pregnant and non-pregnant women

The *total amount of haemoglobin* (THb) in the 10 subjects in whom the external blood loss at delivery was measured, was on the average 106 g less 3–5 days after delivery than before delivery. At delivery and during the first 4 *post partum* days the estimated average external blood loss was 64.6 g Hb. The difference is 42.2 ± 14.6 g Hb ($P < 0.01$).

Discussion

CO production

The COHb saturation reflects the rate of CO production but it is also influenced by such factors as the CO concentration in ambient air and the alveolar ventilation (*Coburn et al.* 1963). During pregnancy and labour hyperventilation and a high O_2 consumption is present (*Cugell, Frank* and *Gaensler* 1953, *Cohen* and *Thomson* 1939). After delivery O_2 consumption and ventilation decrease. Therefore the rate of CO production is best measured directly.

In our series the mean CO production of the pregnant women, 0.59 ml CO per hour, was somewhat higher than that of the healthy non-pregnant controls, 0.39 ml CO per hour. *Coburn et al.* (1963) using a somewhat different method found the mean production in a group of 10 young men to be 0.42 ml CO per hour.

The CO produced by the body originates mainly from the breakdown of Hb (*Coburn et al.* 1964, *Luomanmäki* 1966). It was therefore of interest to study the rate of CO production per g THb. As this was approximately equal in pregnant women during the last month of pregnancy and in non pregnant women it seems likely that the increase in THb during pregnancy accounts for most of the increase of the CO production rate. The CO production per g THb is higher in our groups than that reported by *Coburn, Forster* and *Kane* (1965). The difference is probably due to methodological dissimilarities.

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COAGULATION AND FIBRINOLYSIS IN NORMAL WOMEN IMMEDIATELY POST PARTUM AND IN NEWBORN INFANTS

Influence of Prophylactic Vitamin K

BY

NIELS CHR. NIELSEN

A number of studies have been concerned with coagulation and the fibrinolytic system in the parturient woman and the newborn infant. However, the majority of these studies have included only a very few patients or a few coagulation and fibrinolysis parameters. There has been no major collected study on coagulation as well as fibrinolysis in mothers and their newborn infants.

Previous Investigations

First Phase of Coagulation

Platelet. The platelet count in the peripheral maternal blood immediately after delivery is often reported to be at the lower end of the normal range. *Bellwinkel* (1951) and *Nilsen* (1963) have reviewed the early literature. The findings have later been confirmed by *Skjodt* and *Albrechtsen* (1965).

In cord blood the platelet count is approximately the same as in adults (*Sanford* and *Shmigelsky* 1942, *Larrieu et al.* 1952, *Runge et al.* 1954, and others). *Runge et al.* demonstrated that there is no relationship between the counts in the mother and her infant.

Factor VIII (Antihaemophilic factor, AHF). The content is

and did not change significantly after delivery In some of the pregnant women the total haemoglobin of the body (THb) was determined before and after delivery as well as the external blood loss during and after delivery The decrease in THb was greater than the estimated external loss of haemoglobin. The "hidden blood loss could not be accounted for even partly by increased haemolysis.

An investigation of the specificity of the methods for CO analysis revealed that the palladium molybdenum test tube method for analysis of CO in gas was insensitive to nitrous oxide trichlor ethylene and chloroform. The hopcalite meter was sensitive to nitrous oxide chloroform and probably to trichlorethylene and the infra red meter to nitrous oxide

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Factor VIII (Antihaemophilic factor AHF) The content is

increased in parturient women and normal in cord blood. This was confirmed by *Kasper et al* (1964) who reviewed previous publications.

Factor IX (Christmas factor) is increased in maternal blood (*Fresh et al* 1956 *Ratnoff* and *Holland* 1959 and *Kasper et al* 1964) Other authors *Nilsson* and *Kullander* (1967 a) using haemophilic B plasma as test substrate found small or no increase. The cord blood on the other hand shows a fairly pronounced decrease (*Fresh et al* 1956 *BarLhan* 1957 and *Künzer* and *Ströder* 1957)

Second Phase of Coagulation

Factor II (Prothrombin) For reviews of the literature and assess ment of the various methods for prothrombin determination the reader is referred to *Thordarson* (1941) *Dyggve* (1952) *Larsen* (1952) *Schwen er et al* (1958) *Nilsen* (1963) and *Nilsson* and *Kullander* (1967 a)

In the light of previous reports it may be said that the prothrombin content is either elevated or possibly only slightly affected in the maternal blood and decreased in cord blood when the determinations are carried out by a two-stage technique or by a specific one-stage technique

It is agreed that no relationship is to be found between the prothrombin content of the maternal blood at delivery and the umbilical cord blood *Dyggve* (1952) has reviewed the literature on this aspect.

Factor VII (Proconvertin) The content in the maternal peripheral blood in late pregnancy and at parturition is elevated. A number of reports have been reviewed by *Nilsen* (1963)

Dyggve (1958) referred to a number of studies all of which showed a reduced proconvertin content in cord blood and demonstrated that no relationship exists between the procon vertin content of the maternal blood and the infant s blood.

Factor X (Stuart Prower factor) is increased in the blood of pregnant women (*Pechet* and *Alexander* 1961) whereas in cord blood it is reduced (*Beller* 1957 and *Kün er et al* 1964)

Factor V (Proaccelerin) In late pregnancy and at delivery the

values are generally considered to be normal (*Larrieu et al.*, 1952 *Alexander et al.*, 1956 *Fresh et al.*, 1956 *Beller* 1957 *Schwenzer et al.*, 1958 *Nilsen*, 1963 *Skjodt* and *Albrechtsen*, 1965 and *Nilsson* and *Kullander* 1967 a) However in a small series *Schultze* and *Schwick* (1953 a) demonstrated an increase in factor V levels.

In cord blood the content of factor V is usually found to be normal (*Larrieu et al.* 1952 *Quick et al.* 1952 *Schultze* and *Schwick* 1953 a *Beller* 1957 *Forgács et al.*, 1962, and others) However *Fresh et al.* (1956) and *Vest* and *Meir* (1957) have reported an increased content.

In a few cases where maternal values were elevated, the content in the cord blood was also elevated (*Beller* 1957)

Third Phase of Coagulation

Factor I (Fibrinogen) As early as 1885 it was observed that the fibrinogen content of cord blood was considerably lower than that of the maternal blood (*Krüger* 1886) This observation has been confirmed by a large number of investigations.

Nilsen (1963) reviewed a number of publications reporting increased fibrinogen in the blood during pregnancy

In newborn infants the fibrinogen content is usually at the lower end of the normal range *Taylor* (1957) reviewed a number of early papers and these findings were later confirmed by *Engstrom* and *Kager* (1964) *Markarian et al.* (1967) and others.

Haematocrit Most authors have reported a slightly decreasing haematocrit towards term and an increase *post partum*. Some of the early publications have been reviewed by *Nilsen* (1963)

Cord blood has a higher haematocrit level than adult blood (*Mugrage* and *Andresen* 1936 *Waugh et al.* 1939 and *Gairdner et al.* 1952)

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The different and often conflicting results published on the fibrinolytic components during pregnancy and delivery as well as in newborn infants are due largely to varying test methods and the lability of the active enzyme

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The different and often conflicting results published on the fibrinolytic components during pregnancy and delivery as well as in newborn infants are due largely to varying test methods and the lability of the active enzyme

A large number of studies have revealed a reduced fibrinolytic activity in healthy pregnant women. On the other hand, the activity is increased immediately *post partum*. *Nielsen* (1963) reviewed the literature, but in his own study he could not demonstrate increased fibrinolytic activity in plasma either during or after delivery but in the euglobulin fraction a slight or moderate increase was found. In a recent study *Nilsson* and *Kullander* (1967 a) were not able to demonstrate any increase in the fibrinolytic activity in plasma immediately before and after delivery

The plasminogen content has been mentioned by only a few authors. *Shaper et al.* (1965) and *Brakman* (1966) reported unchanged values during the pregnancy and puerperium, while *Samartzis et al.* (1960) *Skjodt* and *Albrechtsen* (1965) *Nilsson* and *Kullander* (1967 a) and others, stated that the plasminogen content was elevated at the time of delivery

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In cord blood the plasminogen level is reduced (*Cope* and *Mitchell* 1964 *Beller et al* 1966 and others)

Influence of Vitamin K upon the Clotting and Fibrinolytic Factors

As far as the mothers are concerned, only prothrombin has been studied. *Bohlender et al.* (1941) and *Mc Cready et al.* (1942) could not demonstrate any effect of vitamin K, while *Quick et al.* (1952) found longer prothrombin times in parturient women who had not been treated with vitamin K than among those who had

Cord blood has been studied in respect to factors II V and VII.

There is great disagreement concerning the influence of vitamin K, both as far as prothrombin and proconvertin are concerned.

Dyggve (1958) has given an extensive review of the literature on this subject.

Factor V is not affected by vitamin K (*Quick et al.*, 1952, and *Fresh et al.* 1957)

There ha e been no investigations on the influence of vitamin K upon fibrinolysis.

Present Investigations

Material and Methods

Analyses were performed on blood samples from the following three groups

Group 1 Twenty-five normal, non-pregnant women in the age range 19 to 38 years, average 24 years. The blood samples were drawn between 8 and 9 a.m. regardless of the relation to menstrual cycle since this has been shown by *Beller et al.* (1964) and *Brakman et al* (1966) to ha e no influence upon coagulation or fibrinolysis. It was ensured that none of these women was using hormonal contraceptives which may give rise to changes in these coagulation components (*Amris* and *Starup* 1967 *Brakman et al.* 1967 *Nilsson* and *Kullander* 1967 b and others)

This group of normal women will be used below as a basis for comparison of coagulation and fibrinolysis in normal women immediately post partum and in newborn infants.

Group 2 Twenty normal women in whom the samples were drawn within the first half-hour after normal delivery None of these women had received vitamin K

Blood samples were obtained also from these women s newborn infants within the first 20 min after birth. All the infants were full-term, healthy and showed no abnormalities during their stay in the department

Group 3. Twenty normal women and their infants. This group of women received menadione tablets, either 20 mg daily or 40 mg as a single dose between 2 and 24 hours before delivery The results showed no difference between the two dosage schedules.

The blood samples from the adults were drawn from a cubital in after application of a light tourniquet. The ein was punctured with a siliconized disposable needle and the samples were

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used only if the puncture proved easy The blood was collected into ordinary glass tubes (14 × 100 mm) and into Owren s plastic thrombotest tubes As anticoagulant sodium citrate 3 8 % was used at the ratio 1 10 except in the haematocrit determinations where dried potassium ammonium oxalate was employed.

Blood collected into plastic tubes was centrifuged immediately at 1500 r p.m. for 5 min (platelet rich plasma) and analysed immediately

Blood collected into glass tubes was centrifuged for 30 min. at 3500 r p.m. (platelet-poor plasma) whereupon the plasma was frozen and stored at —20 C for later analysis

From the newborn infants blood was drawn by the following technique Immediately after birth the cord was cut after applica tion of *Hesseltine s* clamp (1937) about 5 cm from the skin. The cord was incised and a disposable plastic catheter was passed to the umbilical vein. The sample was used only if there was a free flow through the catheter

The analyses stated below were performed using the platelet rich plasma for item (1) and (2) and the platelet poor plasma for the rest.

All the results except those of the fibrinolytic activity and the thromboplastin activation test, are given as the mean of two analyses The fibrinolysis values are the mean of three determinations.

(1) Recalcification time in sec., in a mixture of 2 ml veronal buffer 0.25 ml 0 025 M $CaCl_2$ and 0.25 ml plasma, performed in a Owren thrombotest plastic tube

(2) Thromboplastin activation test (two-stage TAT) as a modified *Ollendorff* and *Astrup* procedure (1966) using plastic instead of silicontzed glass. This invstigation concerns the formation of plasma thromboplastin and thrombin in the plasma following recalcification. The result is shown in a diagram in which the abscissa is the incubation period in min. and the ordinate the log of the clotting time in sec This diagram is characterized by the following values t_1 t_7 T max and t max as well as the recalcification time (*cf* Fig 1) Combined with the other coagulation analyses the two-stage TAT tells whether the thromboplastin activity is increased. However this particular factor is better elucidated by a modification of the test viz three-stage TAT

In principle the three-stage TAT serves to demonstrate any cl t-promoting components in the test plasma by determining its coagulation-activating effect upon normal platelet-rich human plasma.

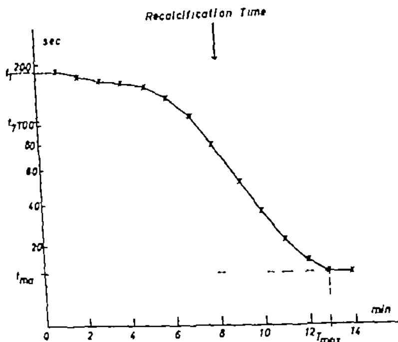


Fig 1 Thromboplastin activation curve of peripheral blood from 25 normal women The curve is characterized by the following values

1 Recalcification time in the incubation mixture in seconds (marked by arrow)

2 t Coagulation time in the first tube, one minute after recalcification of the incubation mixture.

3 t_7 Coagulation time in the seventh tube 7 minutes after recalcification of the incubation mixture.

4 T max The number of minutes until the shortest clotting time is obtained.

5 t max The clotting time in seconds at Tmax.

The incubation mixture consisted of 2 0 ml veronal buffer + 0 20 ml normal platelet-rich human plasma 0 05 ml platelet-rich test plasma 0 25 ml 0 025 M $CaCl_2$

(3) Partial thromboplastin time (PTT) in sec according to the method described by *Nye et al* (1962) Thrombofax Ortho® was used as the partial thromboplastin reagent

(4) Thrombin time by determination of the clotting time in sec In a mixture of 0 1 ml plasma, 0 1 ml veronal buffer and 0 1 ml thrombin solution (20 NIH/ml) at 37° C

(5) Prothrombin time in sec by the method of Quick (1935).

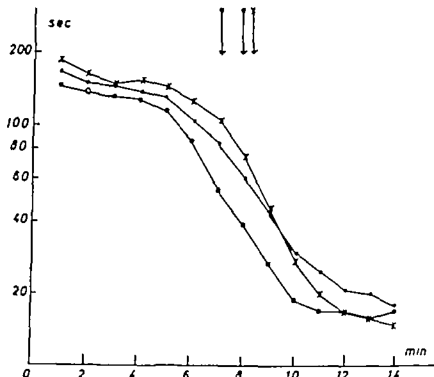


Fig. 2. Thromboplastin activation curve of peripheral blood from 20 normal women immediately after delivery. None hade received vitamin K.
×—× 15 normal adult women, two-stage TAT
●—● 20 normal women immediately after delivery two-stage TAT
○—○ 20 normal women immediately after delivery three-stage TAT

(6) Factor V measured in per cent of normal as described by *Biggs* and *Macfarlane* (1965)

(7) Prothrombin-proconvertin in per cent of normal (PP) by the method of *Owren* and *Aas* (1951)

(8) Fibrinogen measured in mg/100 ml citrated plasma as advocated by *Sl.jodt* and *Albrechtsen* (1965). The result was corrected for the dilution with sodium citrate

(9) Plasminogen measured in *ug* copper tyrosine released per ml activated plasma (*Alkjærsig et al* 1959)

(10) Fibrinolytic activity determined on standard fibrin plates (*Astrup* and *Mullertz* 1952) and on heated fibrin plates (*Lassen* 1952) The plates were produced from bovin fibrinogen 0 2 % Povite®

Every heated plate was checked by the plasminogen activator urokinase to make sure that all the plasminogen had been destroyed by the heating.

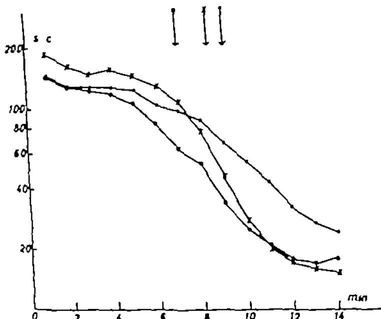


Fig 3 Thromboplastin activation curve of venous cord blood from 20 newborn infants whose mothers had not received vitamin K
×—× 15 normal adult women, two-stage TAT
●—● 20 normal newborn *infants* two-stage TAT
⌒ 20 normal newborn infants, three-stage TAT

The fibrinolytic activity was determined in untreated plasma as well as in the euglobulin fraction after iso-electric precipitation at pH 5.9 (*Astrup* and Rasmussen 1958)

(11) Haematocrit measured in per cent, determined in Wintrobe haematocrit tubes (Wintrobe 1956)

(12) Platelet counts after brief centrifugation of citrate-stabilized blood at 1500 p m The counting was done in Thoma chamber with depth of 0 05 mm, using phase contrast microscope

Results

The results of the *coagulation tests* are listed in Table I The curves representing TAT and the recalcification time are plotted in Figs. 1 2, 3 4 and 5

Table I *Results of Coagulation Analyses*

Tests see Methods	Normal Women (not pregnant)		Normal Women (Immediately after delivery)		Normal Newborn Infants		Prophylactic Administration of Vitamin K: Normal Women (Immediately after delivery)		Prophylactic Administration of Vitamin K: Normal Newborn Infants	
	x̄ and n	range est. SD	x̄ and n	range est. SD	x̄ and n	range est. SD	x̄ and n	range est. SD	x̄ and n	range est. SD
Platelets	352	223 – 498	207	67 – 351	323	66 – 571	221	142 – 383	527	224 – 863
(thousands per cu.mm.)	n = 25	± 77	n = 20	± 76	n = 20	± 147	n = 20	± 70	n = 20	± 207
Proaccelerin	104	85 – 145	110	80 – 190	115	62 – 170	122	75 – 210	122	82 – 160
(per cent)	n = 25	± 16	n = 20	± 31	n = 20	± 34	n = 20	± 37	n = 20	± 30
Prothrombin time	18	15 – 21	17	16 – 20	19	17 – 22	17	14 – 20	19	18 – 21
(seconds)	n = 25	± 1.2	n = 20	± 0.9	n = 20	± 1.5	n = 20	± 1.4	n = 20	± 0.9
PP	103	76 – 120	206	120 – 380	72	30 – 115	227	165 – 340	90	62 – 150
(per cent)	n = 25	± 13	n = 20	± 68	n = 20	± 25	n = 20	± 51	n = 20	± 21
Partial thromboplastin time	81	69 – 100	73	66 – 79	88	78 – 125	75	65 – 81	92	79 – 135
(seconds)	n = 25	± 5.9	n = 20	± 3.6	n = 20	± 11	†n = 19	± 4.0	n = 20	± 12
Thrombin time	9	7 – 12	8	5 – 10	13	10 – 15	8	5 – 10	14	9 – 19
(seconds)	n = 25	± 1.0	n = 20	± 1.1	n = 20	± 1.5	n = 20	± 1.6	n = 20	± 2.5
Fibrinogen	271	165 – 360	508	320 – 985	242	150 – 340	502	380 – 690	239	130 – 345
(mg. per 100 ml)	n = 25	± 45	n = 20	± 154	n = 20	± 50	n = 20	± 88	n = 20	± 51
Haematocrit	40	34 – 46	42	36 – 48	55	48 – 63	41	34 – 46	56	46 – 62
(per cent)	n = 25	± 3.1	n = 20	± 3.2	n = 20	± 4.9	n = 20	± 3.2	n = 20	± 3.6

$\bar{x}$ = arithmetic average n = number of estimations

range = range of individual results (x) est. SD = estimated standard deviation = $\pm \sqrt{\frac{\sum (x-\bar{x})^2}{n-1}}$

Clotting times shorter than 60 seconds in the partial thromboplastin time test re not included in the calculations They were bserved in one sample †

Table II Results of Fibrinolysis Analyses

Tests, see Methods	Normal Women (not pregnant)		Normal Women (immediately after delivery)		Normal Newborn Infants		Prophylactic Administration of Vitamin K: Normal Women (immediately after delivery)		Prophylactic Administration of Vitamin K: Normal Newborn Infants	
	x̄ and n	range est. SD	x̄ and n	range est. SD	x̄ and n	range est. SD	x̄ and n	range est. SD	x̄ and n	range est. SD
Standard fibrin plates Untreated plasma (sq mm)	0 25	0 ± 0	0 20	0 ± 0	86 20	16 230 ± 56	0 20	0 ± 0	75 n = 20	26 – 245 ± 51
Standard fibrin plates Euglobulins (sq mm)	12 25	0 36 ± 10	30 20	0 105 ± 25	390 = 20	251 658 ± 119	37 = 20	11 – 75 ± 17	283 n = 20	107 – 576 ± 155
Heated fibrin plates Untreated plasma (sq mm)	0 25	0 ± 0	0 20	0 ± 0	25 20	9 – 49 ± 9 7	0 n = 20	0 ± 0	25 = 20	4 – 49 ± 10
Heated fibrin plates Euglobulins (sq mm)	12 25	0 32 ± 8 0	27 20	0 49 ± 14	34 20	19 49 ± 7.6	25 20	9 – 78 ± 16	38 = 20	25 – 54 ± 8.2
Plasminogen (µg Co-Tyrosine per ml)	127 n 25	78 172 ± 23	173 = 20	108 234 ± 25	67 20	37 108 ± 19	168 = 20	126 – 208 ± 21	61 n = 20	35 – 104 ± 19

See footnote to Table I.

Table I. *Results of Coagulation Analyses*

Tests see Methods	Normal Women (not pregnant)		Normal Women (immediately after delivery)		Normal Newborn Infants		Prophylactic Administration of Vitamin K: Normal Women (immediately after delivery)		Prophylactic Administration of Vitamin K: Normal Newborn Infants	
	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD	$\bar{x}$ and n	range est. SD
Platelets	352	223 – 498	207	67 – 351	323	86 – 571	221	142 – 383	527	224 – 863
(thousands per cu mm)	n = 25	± 77	n = 20	± 76	n = 20	± 147	n = 20	± 70	n = 20	± 207
Proaccelerin	104	85 – 145	110	80 – 190	115	62 – 170	122	75 – 210	122	82 – 160
(per cent)	n = 25	± 16	n = 20	± 31	n = 20	± 34	n = 20	± 37	n = 20	± 30
Prothrombin time	18	15 – 21	17	16 – 20	19	17 – 22	17	14 – 20	19	18 – 21
(seconds)	n = 25	± 1 2	n = 20	± 0.9	n = 20	± 1.5	n = 20	± 1 4	n = 20	± 0.9
PP	103	76 – 120	206	120 – 380	72	30 – 115	227	165 – 340	90	62 – 150
(per cent)	= 25	± 13	n = 20	± 68	n = 20	± 25	n = 20	± 51	n = 20	± 21
Partial thromboplastin time	81	69 – 100	73	66 – 79	88	78 – 125	75	65 – 81	92	79 – 135
(seconds)	n = 25	± 5 9	n = 20	± 3.6	n = 20	± 11	†n = 19	± 4 0	n = 20	± 12
Thrombin time	9	7 – 12	8	5 – 10	13	10 – 15	8	5 – 10	14	9 – 19
(seconds)	n = 25	± 1.0	n = 20	± 1 1	n = 20	± 1.5	n = 20	± 1 6	n = 20	± 2.5
Fibrinogen	271	185 – 360	508	320 – 985	242	150 – 340	502	380 – 690	239	130 – 345
(mg. per 100 ml)	n = 25	± 45	n = 20	± 154	n = 20	± 50	n = 20	± 88	n = 20	± 51
Haematocrit	40	34 – 46	42	36 – 48	55	48 – 63	41	34 – 46	56	46 – 62
(per cent)	n = 25	± 3 1	n = 20	± 3.2	n = 20	± 4 J	n = 20	± 3 2	n = 20	± 3 6

$\bar{x}$ = arithmetic average n = number f estimations

range = ange of individual results (x) est. SD = estimated tandard devi tion = ± $\sqrt{\frac{\Sigma (x-\bar{x})^2}{n-1}}$

Clotting times shorter than 60 seconds in the partial thromboplastin time test re not included i the calcul tions They were bserved in one sample †

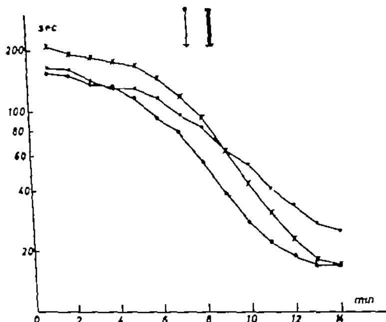


Fig. 5 Thromboplastin activation curve of venous cord blood from 20 newborn infants whose mothers had received vitamin K.
— 20 normal adult women, two-stage TAT
●—● 20 normal newborn infants, two-stage TAT
○—○ 20 normal newborn infants, three-stage TAT

PP indicates distinctly that prothrombin-proconvertin, and possibly also the included factor X content, were significantly increased ($p < 0.001$)

The fibrinogen content was significantly increased ($p < 0.001$) and the thrombin time shortened ($p < 0.001$)

The haematocrit was unchanged.

B *Cord blood* The platelet count was unchanged.

Two-stage TAT showed a significant shortening of t_1 ($p < 0.001$) but no change of t and T max, while a significant prolongation of t max ($p < 0.001$) and of the recalcification time ($0.02 > p > 0.01$)

Three-stage TAT showed the same changes as for the mothers, i.e. a significant shortening of t_1, t_2 and of the recalcification time ($p < 0.001$) but no change of T max or t max.

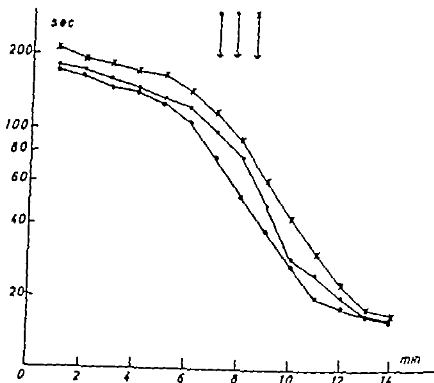


Fig. 4 Thromboplastin activation curve of peripheral blood from 20 normal women immediately after delivery Prophylactic administration of vitamin K
×—× 20 normal adult womeen two-stage TAT
●—● 20 normal women immediately after delivery two-stageTAT
○—○ 20 normal women immediately after delivery three stage TAT

Comparison of the values found in normal non-pregnant women (group 1) and in normal women immediately post partum as well as in their newborn infants (group 2) showed the following

A. *Peripheral maternal blood* A significant reduction of platelet count ($p < 0\ 001$)

Two-stage TAT revealed a significant shortening of t_1 ($0\ 01 > p > 0\ 001$) and of the recalcification time ($0.05 > p > 0\ 02$) and a tendency to shortening of t_7 On the other hand, T max and t max were unchanged.

Three-stage TAT showed a significant shortening of t_1 t_7 and of the recalcification time ($p < 0\ 001$) but no change in T max or t max.

A significant shortening of PTT ($p < 0\ 001$) and of the Quick time ($0\ 05 > p > 0\ 02$) while factor V remained unchanged

Comparison of group 2 and 3 shows that vitamin K has no influence upon the clotting and fibrinolysis factors in the *peripheral maternal blood*

In *cord blood* the platelet count was significantly higher in the group where the mothers received vitamin K ($0.01 > p > 0.001$)

The PP in the group where the mothers did not receive vitamin K was significantly lower than in the group treated prophylactically with this vitamin ($0.05 > p > 0.02$)

Vitamin K exerted no influence upon fibrinolysis.

Discussion

Coagulation

In the *peripheral maternal blood* there was a reduction of platelet count, but otherwise an increased content of the first-phase coagulation factors as expressed by the two-stage TAT This is also compatible with a shortened PTT which is, essentially a screening test for the entire coagulation mechanism, except for factor VII. On the basis of these two tests it is not possible to decide whether the increase is in factor VIII or factor IX—or perhaps both.

The three-stage TAT showed a significant shortening of t_1, t_2 as well as of the recalcification time This must be taken to indicate that after normal delivery the peripheral maternal blood contains clot-promoting components.

In premature separation of the placenta and at times also in eclampsia *Schneider* (1951) demonstrated that thromboplastin, presumably of tissue origin, passes from the retroplacental or intradecidual haematoma to the maternal circulation leading to intravascular coagulation. It might be imagined that the same occurs in association with normal delivery only to a much

Being suspected that the increased platelet count of newborn children, whose mothers had received prophylactic vitamin K, might be due to systematic error re-investigations have been made on another group of newborn children These investigations proved that the platelet count in the umbilical cord blood was independent of the prophylactic vitamin K received by the mothers

A significant prolongation of PTT ($0.02 > p > 0.01$) and of the Quick time ($0.05 > p > 0.02$) while factor V was unchanged, and PP significantly reduced ($p < 0.001$)

The fibrinogen content showed a tendency to be reduced, while the thrombin time was significantly prolonged ($p < 0.001$)

The haematocrit was significantly elevated ($p < 0.001$)

The result of the *fibrinolysis studies* is listed in Table II. Comparison of group 1 with group 2 showed the following:

A. *Peripheral maternal blood* Plasminogen content was found to be significantly elevated ($p < 0.001$) Fibrinolytic activity could not be demonstrated, either on standard fibrin plates or on heated plates. This indicates the absence of activator activity as well as of circulating plasmin. In the euglobulin fraction on the other hand, there was a significant increase in fibrinolytic activity on standard fibrin plates as well as on heated plates ($p < 0.001$)

This difference between the fibrinolytic activity in precipitated and non-precipitated plasma which is significant ($p < 0.001$) may presumably be taken to indicate the presence of inhibitors in the plasma.

B *Cord blood* Plasminogen content was significantly reduced ($p < 0.001$) Increased fibrinolytic activity was found in non-precipitated plasma on standard as well as on heated fibrin plates ($p < 0.001$) indicating the presence of activator-activity as well as of circulating plasmin. In the euglobulin fraction there was also a significant increase ($p < 0.001$) in fibrinolytic activity on standard as well as on heated fibrin plates. This finding too must be taken to indicate the presence of activator activity as well as of plasmin.

When considering the difference in fibrinolytic activity between non-precipitated and precipitated plasma, the activity is significantly higher in the euglobulin fraction ($p < 0.001$) As stated above this may indicate the presence of inhibitors in the plasma.

Influence of Vitamin K upon the Clotting and Fibrinolysis Factors

Tables I and II give the results of the coagulation and fibrinolysis tests in cases where the mothers received prophylactic vitamin K (group 3)

As far as the 2nd and 3rd phases of coagulation are concerned, the results agree with those of previous authors.

The prolonged thrombin time in cord blood, combined with the modest reduction of fibrinogen, must be taken to indicate the presence of antithrombin. This also accords with the prolonged t max in the two-stage TAT. To investigate this aspect in more detail, the following investigations were carried out after the completion of the main study.

In 10 samples of cord blood the thrombin time was found to average 12.3 sec. By replacing veronal buffer by toluidine blue, it was not possible to normalize the prolonged thrombin time, the mean value for the 10 tests being 11.1 sec. Thus, it may be established that the antithrombin present is not of heparin nature, but possibly it may have been formed as a consequence of the modest intravascular coagulation or of the presence of fibrinolytic split products.

The result of the haematocrit determinations agrees with previous findings.

Fibrinolysis

It is apparent from the results found in elucidating fibrinolysis that an essential difference exists between the findings in mothers immediately post partum and in their newborn infants.

In the *peripheral maternal* blood the enzyme pre-stage plasminogen was significantly increased. In the euglobulin fraction there was a significant increase in activator-activity and in plasmin. Since this did not manifest itself in fibrinolytic activity in non-precipitated plasma, it may be assumed that the inhibitors have been sufficiently increased to balance with the fibrinolytic system at a higher level.

In cord blood the plasminogen content was significantly reduced. The reason may be either reduced production or increased consumption.

A reduced production is indicated by the experiments of *Beller et al.* (1966) who gave the mothers infusions of EACA immediately before delivery. Although this substance passed the placental barrier, there was no increase in the plasminogen content in the newborns.

slighter extent. The failure to find the TAT curve so characteristic of the presence of tissue thromboplastin (*Astrup* and *Ollendorff* 1961) may be due to disappearance of the tissue thromboplastin immediately after the intravascular coagulation (*Astrup* and *Albrechtsen* 1968 personal communication)

Wessler (1955) has demonstrated that serum possesses a pronounced clot promoting activity "serum thrombotic accelerator" Thus it is possible that the demonstrated clot-promoting component is identical with *Wessler's* serum factor which may have been released by the intravascular coagulation resulting from the tissue thromboplastin.

For the 2nd phase of coagulation the results are in agreement with previous findings. The reason why it is difficult to measure minor fluctuations in the prothrombin content by the Quick method is that the Quick time is a screening test which determines factors II V VII and possibly also X. Indeed, it has been demonstrated that an elevated factor V content may exert a normalizing effect upon the Quick time so that real reductions in prothrombin do not manifest themselves (*Owren* 1950 *Schultze* and *Schwick* 1953 b and *Ferguson* and *Patch* 1956)

The results of the fibrinogen determinations, the 3rd phase of coagulation are also in accordance with the results of previous investigations and this also applies to the haematocrit determinations.

In *cord blood* t_1 was shortened in the two-stage TAT while the recalcification time was prolonged. The result of the three-stage TAT which demonstrated distinctly the presence of clot promoting components with shortened t_1, t_7 and recalcification time indicates a reduced content of the 1st-phase clotting factors. Indeed, this is also compatible with a prolonged PTT

In view of previous findings it must be assumed that there is only a question of a reduction of factor IX.

Thus it is possible that clot-promoting components pass from the maternal to the infant's blood during delivery This possibly explains why many investigators have found shortened clotting times for whole blood in spite of reduction of several coagulation factors.

6. Fibrinogen was greatly increased in the mothers while in the infants there was a tendency to reduction.
7. No fibrinolytic activity was detected in the plasma of the mothers, but a highly increased fibrinolytic activity with circulating plasmin, in the newborn.
8. The plasminogen content was considerably elevated in the mothers, but considerably reduced in the infants.
9. The haematocrit was unchanged in the mothers, but elevated in the infants.
10. Vitamin K has no influence upon maternal coagulation. On the other hand, the group of newborn infants whose mothers had received vitamin K showed a higher prothrombin-proconvertin content than the group whose mothers had not received this prophylaxis.
11. Vitamin K has no influence upon the fibrinolytic components, either in the mothers or in the newborn infants.

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As is apparent from the results in Table II there must, however also be a question of an increased consumption, as there was a brisk fibrinolytic activity with circulating plasmin.

The cause of this brisk fibrinolytic activity must be assumed to be the effect upon the infant during delivery as well as the reduction in the pH of the infant's blood immediately after birth (*Engstrom* and *Kager* 1964) However it is possible also that there may be a question of intravascular coagulation with secondary fibrinolysis.

Influence of Vitamin K upon the Coagulation and Fibrinolysis Factors

The results showed that vitamin K administrated to the mother before delivery has no influence upon the coagulation in the peripheral maternal blood immediately post partum. On the other hand, it was found in agreement with a number of previous studies that in the cord blood the PP was lower in newborn infants whose mothers had not received vitamin K than in the group where this prophylaxis had been used.

Vitamin K was not found to affect fibrinolysis either in the peripheral maternal blood or in the cord blood.

SUMMARY

A study of coagulation and fibrinolysis components in normal women immediately post partum and in their newborn infants, including the influence of prophylactic vitamin K upon these components gave the following results

1 The platelet count was reduced in the maternal blood while no definite changes were found in the newborn infants.
2 The two-stage TAT showed the factors in the first stage of coagulation to be increased in the mothers and reduced in the infants.
3 The three-stage TAT showed clot promoting factors in the blood of the mothers as well as of the infants
4 Proaccelerin was unchanged in mothers as well as infants.
5 The prothrombin-proconvertin content was greatly elevated in the mothers, but reduced in the infants

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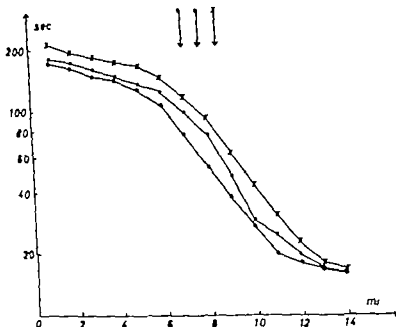


Fig 1 Thromboplastin activation curve of peripheral blood from 20 normal women immediately after delivery Prophylactic administration of vitamin K.
– 20 normal adult women, two-stage TAT
●—● 20 normal women immediately after delivery two-stage TAT
( ) 20 normal women immediately after delivery three-stage TAT

in both categories the values are higher than in normal non-pregnant women Newborn infants of diabetic mothers and premature infants of the same age (36–37 weeks) show no differences in fibrinolytic activity or plasminogen content, but if both groups are compared with normal full-term infants, the plasminogen content is found to be lower while the fibrinolytic activity is in the same range

Ambrus et al (1963) found the plasminogen content in five newborn of diabetic mothers to be in the same range as in normal full term or premature infants.

Aarhus Nich Chr Acta obst. et gynec. scandinav 48, 392, 1969

From the University Maternity Hospital (Professor M. Ingerslev) Aarhus Denmark

COAGULATION AND FIBRINOLYSIS IN DIABETIC WOMEN IMMEDIATELY POST PARTUM AND IN THEIR NEWBORN INFANTS

Influence of Caesarean Section

BY

NIELS CHR. NIELSEN

In a previous study the author demonstrated various changes in coagulation and fibrinolysis in normal women immediately *post partum* and in their infants (*Nielsen* 1969) This confirmed in several respects the findings of others.

The object of the present study was to elucidate coagulation and fibrinolysis in diabetic women and their infants immediately post partum

Since empirically diabetic women are not infrequently delivered by Caesarean section the study also included an investigation into the possible influence of this procedure upon the coagulation and fibrinolysis parameters

Previous Investigations

To the author s knowledge there have been no reports on coagulation in pregnant or parturient diabetics or their infants.

Studies on the haematocrit value in the cord blood of infants born to diabetic women have given conflicting results *Nicolopoulos* and *Smith* (1961) found normal but *Probst* (1967) elevated values.

Plasminogen and fibrinolytic activity in the serum are identical in normal and diabetic parturient women (*Samartzis et al* 1960)

Table 1. *Results of Coagulation and Fibrinolysis Studies in Women Immediately Post Partum*

Tests see Methods	Normal Women		Women with Diabetes Mellitus			
			Vaginal Delivery		Caesarean Section	
	x̄ and n	range est. SD	x̄ and n	range est. SD	x̄ and n	range est. SD
Platelets	221	142–383	223	161–350	213	154–290
(thousands per cu mm)	n = 20	± 70	n = 10	± 57	n = 10	± 41
Proaccelerin	122	75–210	120	77–185	110	69–200
(per cent)	n = 20	± 37	n = 10	± 31	n = 10	± 38
Prothrombin time	17	14–20	17	16–20	18	15–20
(seconds)	n = 20	± 1.4	n = 10	± 1.2	n = 10	± 1.4
PP	227	165–340	186	140–255	160	130–210
(per cent)	n = 20	± 51	n = 10	± 37	n = 10	± 30
Partial thromboplastin	75	65–81	75	70–81	76	68–84
time (seconds)	†n = 19	± 4.0	n = 10	± 4.2	n = 10	± 4.7
Thrombin time	8	5–10	7	4–8	8	5–11
(seconds)	n = 20	± 1.6	n = 10	± 1.4	n = 10	± 2.6
Fibrinogen	502	380–690	564	435–690	490	380–775
(mg per 100 ml)	n = 20	± 88	n = 10	± 86	n = 10	± 116
Haematocrit	41	34–46	47	41–50	42	35–46
(per cent)	n = 20	± 3.2	n = 10	± 3.6	n = 10	± 3.8
Standard fibrin plates	0	0	0	0	0	0
Untreated plasma (sq mm)	n = 20	± 0	n = 10	± 0	n = 10	± 0
Standard fibrin plates	37	11–75	27	4–49	15	0–40
Euglobulins (sq mm)	n = 20	± 17	n = 10	± 16	n = 9	± 15
Heated fibrin plates	0	0	0	0	0	0
Untreated plasma (sq mm)	n = 20	± 0	n = 10	± 0	n = 10	± 0
Heated fibrin plates	25	9–78	28	16–38	17	2–36
Euglobulins (sq mm)	n = 20	± 16	n = 10	± 8.9	n = 9	± 11
Plasminogen (µg Co-Tyrosine per ml)	168	126–208	176	144–208	163	121–222
	n = 20	± 21	n = 10	± 18	n = 10	± 32

x̄ arithmetic average n number of estimations

range range of individual results ( ) est SD estimated standard deviation

$$\sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$$

† Clotting times shorter than 60 seconds in the partial thromboplastin time test are not included in the calculations They were observed in one sample†

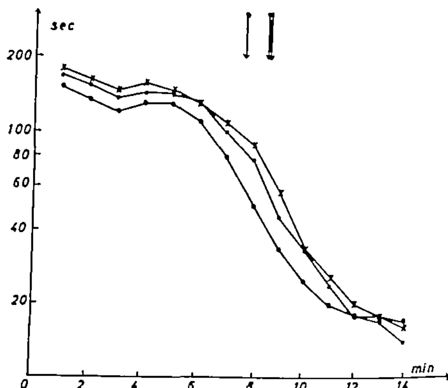


Fig. 2 Thromboplastin activation curve of peripheral blood from 10 women with diabetes mellitus immediately after delivery Caesarean section was not performed.

×—× 9 normal adult women, two-stage TAT

●—● 10 women with diabetes mellitus immediately after delivery two-stage TAT

○—○ 10 women with diabetes mellitus immediately after delivery three-stage TAT

Present Investigations

Material and Methods

The analyses were carried out on blood samples from the following two groups

Group 1 Ten women with diabetes mellitus whose samples were drawn within the first half hour after delivery All had received prophylatic vitamin K in the form of menadione tablets 20 mg daily for about 8 days.

The average duration of diabetes was 5 years (4 months to 12

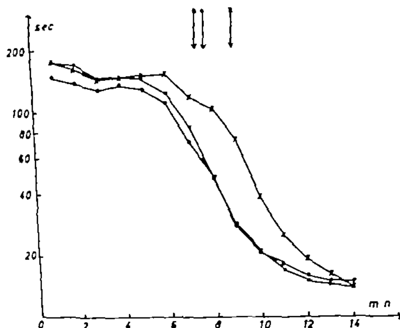


Fig 3 Thromboplastin activation curve of peripheral blood from 10 women with diabetes mellitus immediately after delivery Caesarean section was performed.

10 normal adult women, two-stage TAT

● ● 10 women with diabetic mellitus immediately after delivery two-stage TAT

10 women with diabetes mellitus immediately after delivery three stage TAT

years) No patient exhibited signs of nephropathy All were well-controlled on insulin, and none had ketoacidosis.

Labour was induced about 3 weeks before term by intramuscular injections of Oxytocin followed by rupture of the membranes and an intra enous Oxytocin infusion if necessary All deliveries were by the vaginal route.

Blood samples were also drawn f om these women s newborn infants within the first 20 minutes after birth. The a erage birth weight was 3660 g (2‾00–4700 g) One infant died at 2 days of age of intracranial haemorrhage the others exhibited no complications during their stay in hospital.

Table II *Results of Coagulation and Fibrinolysis Studies in Newborn Infants*

Tests see Methods	Normal Newborn Infants		Newborn Infants of Diabetic Mothers			
			Vaginal Delivery		Caesarean Section	
	x̄ and n	range est. SD	x̄ and n	range est. SD	x̄ and n	range est. SD
Platelets	5[illegible]7	[illegible]4 – 863	253	1[illegible]6 – 425	[illegible]96	57 – [illegible]63
(thousands per cu.mm.)	n = 20	± 207	n = 10	± 93	n = 10	± 142
Proaccelerin	12[illegible]	8 – 160	88	20 – 1[illegible]0	75	[illegible]2 – 113
(per cent)	n = 20	± 30	n = 10	± 35	n = 10	± 30
Prothrombin time	19	18 – 21	[illegible]2	19 – 29	[illegible]1	17 – [illegible]7
(seconds)	n = 20	± 0.9	n = 10	± [illegible]9	n = 10	± 2.4
PP	90	62 – 150	57	31 – 100	46	11 – [illegible]0
(per cent)	n = [illegible]0	± 21	n = 10	± 21	n = 10	± 23
Partial thromboplastin	9[illegible]	[illegible]9 – 135	99	81 – 1[illegible]6	109	83 – 171
time (seconds)	n = [illegible]0	± 1[illegible]	n = 10	± 18	n = 10	± 27
Thrombin time	14	9 – 19	16	11 – 3[illegible]	17	9 – 31
(seconds)	n = 20	± 2.5	n = 10	± 5.8	n = 10	± 6.1
Fibrinogen	239	130 – 345	[illegible]04	90 – [illegible]75	1[illegible]4	90 – 270
(mg per 100 ml.)	n = 20	± 51	n = 10	± 66	n = 10	± 59
Haematocrit	56	46 – 62	66	59 – 71	61	55 – 66
(per cent)	n = 20	± 3.6	n = 10	± 4.1	n = 10	± 3.5
Standard fibrin plates	75	[illegible]6 – 245	56	0 – 144	[illegible]5	0 – 59
Untreated plasma (sq.mm.)	n = 20	± 51	n = 10	± 44	n = 10	± [illegible]3
Standard fibrin plates	283	107 – 5[illegible]6	253	[illegible] – 414	17[illegible]	10 – 373
Euglobulins (sq mm.)	n = 20	± 155	n = 10	± 137	n = 10	± 125
Heated fibrin plates	25	4 – 49	13	0 – [illegible]6	6	0 – 25
Untreated plasma (sq.mm.)	n = 20	± 10	n = 10	± 1[illegible]	n = 10	± 8.8
Heated fibrin plates	38	25 – 54	8	4 – 49	[illegible]4	9 – 36
Euglobulins (sq.mm.)	n = [illegible]0	± 8.2	n = 10	± 14	n = 10	± 9.1
Plasminogen ([illegible]g Cu-Tyrosine per ml.)	61	35 – 104	53	5 – 81	[illegible]4	16 – 66
	n = 20	± 19	n = 10	± 26	n = 10	± 16

See *footnote* to Table I.

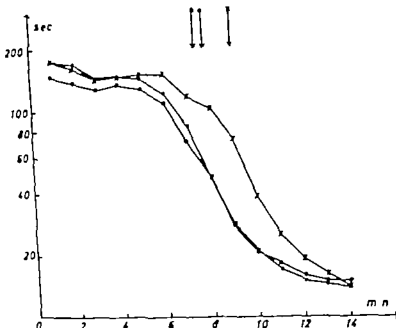


Fig. 3 Thromboplastin activation curve of peripheral blood from 10 women with diabetes mellitus immediately after delivery. Caesarean section was performed.

10 normal adult women, two-stage TAT

● ● 10 women with diabetic mellitus immediately after delivery two-stage TAT

10 women with diabetes mellitus immediately after delivery three-stage TAT

years). No patient exhibited signs of nephropathy. All were well-controlled on insulin, and none had ketoacidosis.

Labour was induced about 3 weeks before term by intramuscular injections of Oxytocin, followed by rupture of the membranes and an intravenous Oxytocin infusion if necessary. All deliveries were by the vaginal route.

Blood samples were also drawn from these women's newborn infants within the first 20 minutes after birth. The average birth weight was 3660 g (2700–4700 g). One infant died at 2 days of age of intracranial haemorrhage; the others exhibited no complications during their stay in hospital.

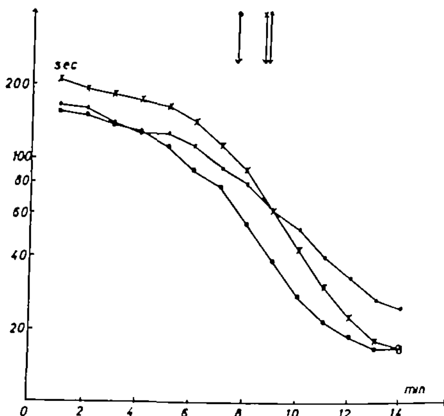


Fig. 4. Thromboplastin activation curve of venous cord blood from 20 newborn infants whose mothers had received vitamin K.
×—× 20 normal adult women, two-stage TAT
●—● 20 normal newborn infants two-stage TAT
○—○ 20 normal newborn infants three-stage TAT

Group 2 Ten diabetic women and their infants. Vitamin K prophylaxis and blood sampling was as described under group 1 (However two patients did not receive vitamin K, as delivery occurred before the prophylaxis had been started. The analytical results in these two cases did not differ from those in the others)

The average duration of diabetes was 8 years (6 months to 14 years) No patient exhibited signs of nephropathy All were on insulin and well-controlled, without signs of ketoacidosis.

Labour was induced about 3 weeks before term except in the two cases where labour started spontaneously All these women were delivered by Caesarean section owing to threatened or mani-

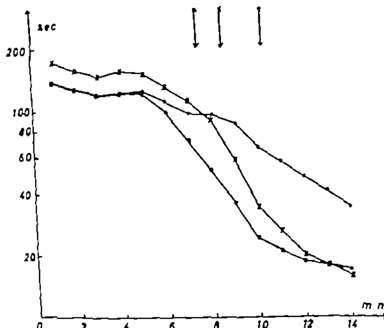


Fig. 5. Thromboplastin activation curve of venous cord blood from 10 newborn infants of diabetic mothers. Caesarean section was not performed.
9 normal adult women, two-stage TAT
● ● 10 newborn infants of diabetic mothers, two-stage TAT
10 newborn infants of diabetic mothers, three-stage TAT

fest complications due to the diabetes. The infants average birth weight was 3350 g (2300–4380 g). One infant died at 8 hours of age and autopsy showed total atelectasis of the lungs. The others showed no complications while in hospital.

Group 3 was a control group of 20 normal parturient women and their newborn infants. Vitamin K was administered and blood samples drawn in the same way as described for the two groups of diabetic women. This control series has been published previously (*Nielsen* 1969).

The technique of blood sampling and further treatment of the samples was as described in the previous paper. The determination of the recalcification time, thromboplastin activation test (TAT), two- and three-stage partial thromboplastin time (PTT), thrombin time, prothrombin time (Quick), factor

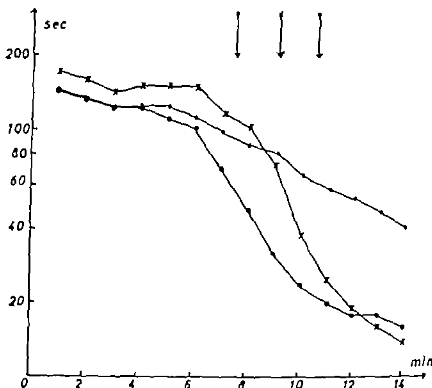


Fig. 6. Thromboplastin activation curve of venous cord blood from 10 newborn infants of diabetic mothers. Caesarean section was performed.
×—× 10 normal adult women, two-stage TAT
●—● 10 newborn infants of diabetic mothers, two-stage TAT
○—○ 10 newborn infants of diabetic mothers three-stage TAT

V prothrombin-proconvertin (PP) fibrinogen content, and determination of fibrinolytic activity in the plasma and in iso-electrically precipitated plasma on untreated fibrin plates (standard plates) and on heated fibrin plates, as well as plasminogen haematocrit, and platelet counts were also done as described in the previous paper (*Nielsen* 1969)

Results

The results of the *coagulation studies* are given in Tables I and II and those of the TAT and recalcification times in Figs. 1 2, 3 4 5 and 6

On comparison between group 1 (diabetic mothers and their infants) and group 3 (normal mothers and their infants) the following observations were made

A. *Maternal peripheral blood.* Apart from the fact that the diabetic had a prolonged recalcification time in TAT two-stage $(0.02 > p > 0.01)$ a reduced PP content $(0.05 > p > 0.02)$ and elevated haematocrit $(p < 0.001)$ there were no significant differences.

B. *Cord blood.* In the infants of diabetic mothers the platelet count was significantly reduced $(p < 0.001)$. TAT two-stage showed a flatter course, with a significantly shortened t_1 $(p < 0.001)$ unchanged t_7 but prolonged t max. There was no difference in T max but a significantly prolonged recalcification time $(0.05 > p > 0.02)$.

TAT three-stage showed, as in the mothers, significant activation, in the same range in the newborn infants of diabetic women as in normal newborns.

In newborn infants of diabetic mothers factor V was significantly reduced $(0.01 > p > 0.001)$, PP reduced $(p < 0.001)$ and the Quick time significantly prolonged $(0.01 > p > 0.001)$.

Otherwise, there were no significant changes in the coagulation parameters. The haematocrit value was significantly higher in the diabetic group $(p < 0.001)$.

The results of the *fibrinolysis studies* are also listed in Tables 1 and 2. Comparison between group 1 and group 3 shows:

A. *Maternal peripheral blood.* No significant difference between the two groups.

B. *Cord blood.* Both groups showed marked fibrinolytic activity in unprecipitated and precipitated plasma, on standard as well as on heated fibrin plates. However, the fibrinolytic activity was more pronounced in normal newborn infants, but this difference was significant only on heated fibrin plates $(0.01 > p > 0.001)$ in untreated plasma, and $(0.02 > p > 0.01)$ in the euglobulin fraction.

Plasminogen content was slightly but not significantly reduced in the diabetic group.

On supplemental examination, confer *Nielse* (1969) this result must be corrected in such way that the conclusion is that there is no difference in the platelet count for children borne by mothers with diabetes mellitus and for normal newborns.

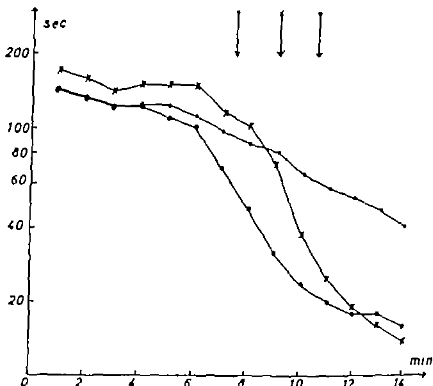


Fig. 6. Thromboplastin activation curve of venous cord blood from 10 newborn infants of diabetic mothers. Caesarean section was performed.
×—× 10 normal adult women, two-stage TAT
●—● 10 newborn infants of diabetic mothers, two-stage TAT
○—○ 10 newborn infants of diabetic mothers three-stage TAT

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On comparison between group 1 (diabetic mothers and their infants) and group 3 (normal mothers and their infants) the following observations were made

and release of serum which, as demonstrated by *Wessler* (1955) possesses a coagulation-promoting activity (*cf* also *Nielsen*, 1969)

The reduced content of coagulation factors in the infants of the diabetic group may be due to an influence of maternal diabetes upon the foetal liver but it may also be a consequence merely of relative prematurity

The greatly elevated haematocrit, which makes the results even more striking, is possibly due to the greatly increased extramedullary haemopoiesis in these infants (*Miller* and *Wilson* 1943)

Fibrinolysis

No difference was found in plasminogen content or fibrinolytic activity between the peripheral blood of *diabetic mothers* and normal mothers or between the *cord blood of infants born to diabetic mothers* compared with that of normal infants.

The highly increased fibrinolytic activity in the cord blood is presumably due to an influence upon the foetus during delivery as well as to the reduction in the pH of the infant's blood immediately after birth (*Engström* and *Kager* 1964) (*Cf* also *Nielsen* 1969)

The brisk fibrinolytic activity which gives rise to the production of an increased quantity of split products perhaps explains the prolonged thrombin time which does not return to normal upon addition of toluidine blue.

Coagulation *and Fibrinolysis Factors Following Caesarean Section*

Caesarean section has no influence upon the coagulation or fibrinolysis factors as judged from samples of peripheral blood drawn from diabetic mothers immediately post partum. Also the cord blood of infants born to diabetic mothers delivered by Caesarean section showed no changes in coagulation factors. On the other hand, the fibrinolytic activity was slightly reduced possibly as a result of a gentler birth.

Coagulation and Fibrinolysis Factors in Patients Delivered by Caesarean Section

On comparison between group 1 (diabetic mothers delivered by the vaginal route and their infants) and group 2 (diabetic mothers delivered by Caesarean section and their infants) the following findings were made in respect to coagulation

A. *Maternal peripheral blood* Apart from a shorter recalcification time ($0.02 > p > 0.01$) and t max ($0.01 > p > 0.001$) in the TAT two stage in group 2 there were no significant differences in coagulation factors The TAT three-stage indicated the same degree of activation in both groups

B *Cord blood* No significant differences between the two groups.

In respect to *fibrinolysis* the two groups showed

A. *Maternal peripheral blood* No significant differences between the two groups.

B *Cord blood* No difference in plasminogen content. On the other hand, fibrinolytic activity was less pronounced in the infants of the group delivered by Caesarean section all the fibrinolysis values being lower than for vaginally delivered infants but the differences are not significant.

Discussion

Coagulation Diabetic mothers proved to have a reduced prothrombin proconvertin level and a prolonged recalcification time as compared with normal mothers These differences are significant. The explanation is presumably that the diabetic mothers were delivered about 3 weeks before term. Other coagulation factors showed no significant differences between the two groups.

The *cord blood of infants born to diabetic mothers* showed compared with that of normal newborns a reduction of all coagulation parameters except for fibrinogen On the other hand the plasma level of coagulation activating components was as in normal infants. The explanation is presumably that at delivery tissue thromboplastin passes to the maternal circulation although only to a slight extent from a small retroplacental or intradecidual haematoma. This leads to intravascular coagulation

Nielsen, Niels Chr. Acta obst. et gynec. scandinav. 48, 403, 1969
From the University Maternity Hospital (Professor M. Ingerslev) Aarhus Denmark

COAGULATION AND FIBRINOLYSIS IN WOMEN DELIVERED BY ELECTIVE CAESAREAN SECTION AND IN THEIR NEWBORN INFANTS

BY

NIELS CHR. NIELSEN

In a previous communication the author reported a finding of coagulation-activating components in the maternal as well as cord blood after normal delivery (*Nielsen* 1969 a). As an explanation it was suggested that small quantities of tissue thromboplastin and serum might pass from a small retroplacental or intradecidual haematoma—similar to *Schneider's* (1951) findings for tissue thromboplastin in abruptio placentae and in eclampsia.

The object of the present study was to elucidate the coagulation and fibrinolysis factors in elective Caesarean section not preceded by labour or by damage to the placenta.

Previous Investigations

To the author's knowledge there have been only a very few reports on coagulation and fibrinolysis in women delivered by elective Caesarean section and in their infants.

Masure and *Schonne* (1956) studying coagulation in 24 newborn infants after normal delivery found hypercoagulability expressed by thrombelastographic changes and a shortened recalcification time in more than half the infants. In five infants delivered by elective Caesarean section the thrombelastography as well as the recalcification time were normal. Investigation of infants delivered by Caesarean section during labour showed the

SUMMARY

A study of the coagulation and fibrinolysis factors in diabetic women immediately post partum and in their infants, including the influence of Caesarean section upon these factors, revealed the following differences from normal mothers and their newborn infants

A. In the maternal blood
   1 Prolonged recalcification time in TAT two-stage, a reduced PP and elevated haematocrit.
   2. No change in fibrinolysis factors

B In cord blood
   1 A flatter course of TAT two-stage with a prolonged recalcification time and t max. Reduced factor V and PP and a prolonged Quick Time. Grealy elevated haemotocrit.
   2. Reduced fibrinolytic activity on heated fibrin plates.

C No influence of Caesarean section upon maternal coagulation or fibrinolysis factors. Among the newborn infants, on the other hand the infants of diabetic mothers delivered by Caesarean section showed less fibrinolytic activity

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2. Reduced fibrinolytic activity on heated fibrin plates.

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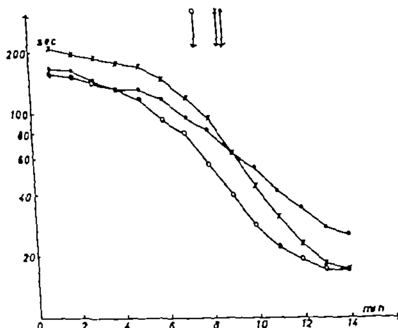


Fig. 2 Thromboplastin activation curve of venous cord blood from 20 normal newborn infants immediately after normal vaginal delivery

— 20 normal adult women, two-stage TAT

●—● 20 normal newborn infants, two-stage TAT

○ 20 normal newborn infants three-stage TAT

section at term by a transverse incision into the uterus. The blood samples were drawn within the first 20 minutes after removal of the placenta. The indication for the operation was usually previous Caesarean section or mechanical disproportion. In no case was it done because of pregnancy complications such as preeclampsia, abruptio placentae etc.

After premedication with atropine, the operation was carried out under general anaesthesia, induced by barbiturate, suxamethonium chloride and intubation, and maintained by nitrous oxide, cyclopropane and oxygen. During the procedure glucose was infused, but never blood prior to the removal of the blood samples.

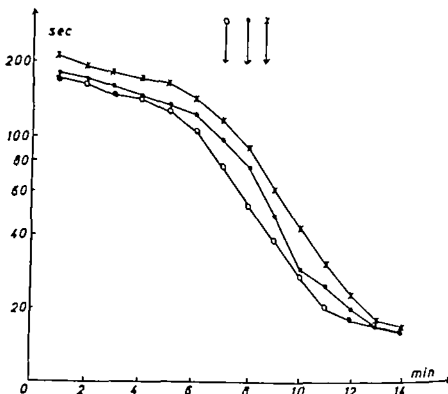


Fig 1 Thromboplastin activation curve of peripheral blood from 20 normal women immediately after normal vaginal delivery
×—× 20 normal adult women two-stage TAT
●—● 20 normal women immediately after delivery two-stage TAT
○—○ 20 normal women immediately after delivery three-stage TAT

same thrombelastographic changes as following normal vaginal delivery It was concluded that the tendency to the hypercoagulability in newborn infants was due mainly to thromboplastic factors of placental origin, liberated in the foetal circulation during labour

Present Investigations

Material and Methods

The analyses were performed on blood samples from the following two groups.

Group I Fifteen normal women delivered by elective Caesarean

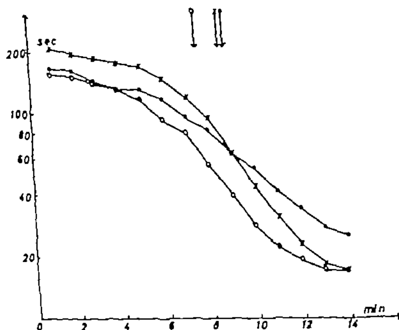


Fig. 2 Thromboplastin activation curve of venous cord blood from 20 normal newborn infants immediately after normal vaginal delivery
— 20 normal adult women, two-stage TAT
●—● 20 normal newborn infants two-stage TAT
○ 20 normal newborn infants, three-stage TAT

section at term by a transverse incision into the uterus. The blood samples were drawn within the first 20 minutes after removal of the placenta. The indication for the operation was usually previous Caesarean section or mechanical disproportion. In no case was it done because of pregnancy complications such as pre-eclampsia, abruptio placentae, etc.

After premedication with atropine the operation was carried out under general anaesthesia, induced by barbiturate, suxamethonium chloride, and intubation, and maintained by nitrous oxide cyclopropane and oxygen. During the procedure glucose was infused but never blood prior to the removal of the blood samples.

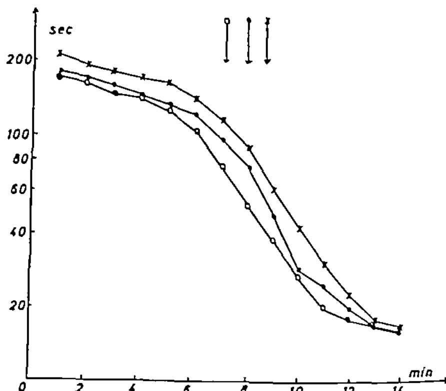


Fig. 1 Thromboplastin activation curve of peripheral blood from 20 normal women immediately after normal vaginal delivery
×—× 20 normal adult women, two-stage TAT
●—● 20 normal women immediately after delivery two-stageTAT
○—○ 20 normal women immediately after delivery three-stage TAT

same thrombelastographic changes as following normal vaginal delivery *It was concluded that the tendency to the hypercoagulability* in newborn infants was due mainly to thromboplastic factors of placental origin, liberated in the foetal circulation during labour

Present Investigations

Material and Methods

The analyses were performed on blood samples from the following two groups.

Group 1 Fifteen normal women delivered by elective Caesarean

Table II. *Results of Coagulation and Fibrinolysis Studies in Normal Newborn Infants*

Tests, see Methods	Normal Newborn Infants			
	Vaginal Delivery		Elective Caesarean section	
	x̄ and n	range est. SD	x̄ and n	range est. SD
Platelets	527	224 – 863	367	225 – 522
(thousands per cu mm.)	– 20	± 207	n = 15	± 84
Proaccelerin	122	82 – 160	104	72 – 143
(per cent)	n = 20	± 30	n = 15	± 20
Prothrombin time	19	18 21	20	18 – 23
(seconds)	n = 20	± 0.9	n = 15	± 1 4
PP	90	62 150	81	62 – 96
(per cent)	n = 20	± 21	n = 15	± 11
Partial thromboplastin time	92	79 135	96	83 – 106
(seconds)	– 20	± 12	– 15	± 8.5
Thrombin time	14	9 – 19	14	9 – 23
(seconds)	n 20	± 2.5	– 15	± 3 5
Fibrinogen	230	130 – 345	216	150 – 315
(mg per 100 ml)	– 20	± 51	– 15	± 49
Haematocrit	56	46 – 62	53	46 – 60
(per cent)	n 20	± 3 6	– 15	± 4 1
Standard fibrin plates	75	26 245	32	0 – 140
Untreated plasma (sq mm)	20	± 51	– 15	± 32
Standard fibrin plates	283	107 576	167	11 – 469
Euglobulins (sq mm)	n 20	± 151	n = 15	± 129
Heated fibrin plates	25	4 – 49	14	0 25
Untreated plasma (sq mm)	– 20	± 10	n = 15	± 10
Heated fibrin plates	38	25 54	23	0 – 40
Euglobulins (sq mm)	20	± 8 2	n = 15	± 12
Plasminogen (ug Cu-Tyrosine per ml)	61	35 104	63	34 110
	20	± 19	n = 15	± 21

See footnote to Table I

Table I. *Results of Coagulation and Fibrinolysis Studies in Women Immediately Post Partum*

Tests see Methods	Normal Women			
	Vaginal Delivery		Elective Caesarean section	
	$\bar{x}$ and n	range est.SD	$\bar{x}$ and n	range est.SD
Platelets	221	147 – 393	229	155 – 296
(thousands per cu.mm.)	n = 20	± 70	n = 15	± 44
Proaccelerin	1[illegible]2	75 – 210	103	71 – 130
(per cent)	n = 20	± 37	n = 15	± 18
Prothrombin time	17	14 – 20	18	15 – 21
(seconds)	n = 20	± 1.4	n = 15	± 1.5
PP	227	165 – 340	186	140 – 240
(per cent)	n = 20	± 51	n = 15	± 20
Partial thromboplastin time	75	65 – 81	75	69 – 82
(seconds)	†n = 19	± 4.0	n = 15	± 3.5
Thrombin time	8	5 – 10	8	6 – 10
(seconds)	n = 20	± 1.6	n = 15	± 0.6
Fibrinogen	502	380 – 690	473	345 – 60
(mg per 100 ml)	n = 20	± 88	n = 15	± 121
Haematocrit	41	34 – 46	39	31 – 47
(per cent)	n = 20	± 3.2	n = 15	± 4.6
Standard fibrin plates	0	0	0	0
Untreated plasma (sq mm.)	n = 20	± 0	n = 15	± 0
Standard fibrin plates	37	11 – 75	16	0 – 79
Euglobulins (sq.mm.)	n = 20	± 17	n = 15	± 20
Heated fibrin plates	0	0	0	0
Untreated plasma (sq.mm.)	n = 20	± 0	n = 15	± 0
Heated fibrin plates	25	9 – 78	17	0 – 51
Euglobulins (sq.mm.)	n = 20	± 16	n = 15	± 14
Plasminogen (µg Cu-Tyrosine per ml)	169	126 – 208	173	150 – 206
	n = 20	± 21	n = 15	± 14

$\bar{x}$ = arithmetic average n = number of estimations
range = range of individual results (x) est SD = estimated standard deviation

$$= \pm \sqrt{\frac{\Sigma (x - \bar{x})^2}{n - 1}}$$

† Clotting times shorter than 60 seconds in the partial thromboplastin time test are not included in the calculations. They were observed in one sample†

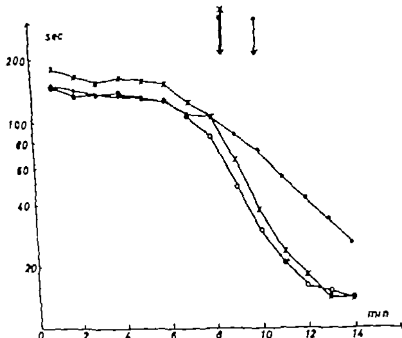


Fig. 4. Thromboplastin activation curve of venous cord blood from 15 normal newborn infants delivered by electiv. Caesarean section.

— 14 normal adult women, two-stage TAT.

●—● 15 normal newborn infants delivered by electiv. Caesarean section, two-stage TAT.

○—○ 15 normal newborn infants delivered by electiv. Caesarean section, three-stage TAT.

Group 2. This was a control group of 20 normal parturient women and their newborn infants. Vitamin K was administered and blood samples drawn just as in group 1. This control group has been described previously (*Nielsen* 1969 a).

The technique of the blood sampling and the further study of the samples were as described previously. The determination of recalcification time, the procedures of the thromboplastin activation test (TAT), two- and three-stage partial thromboplastin time (PTT), thrombin time, prothrombin time (Quick), factor V, prothrombin-proconvertin (PP), fibrinogen content, and the determination of fibrinolytic activity in the plasma and in iso-electrically precipitated plasma on untreated fibrin plates (standard plates) and on

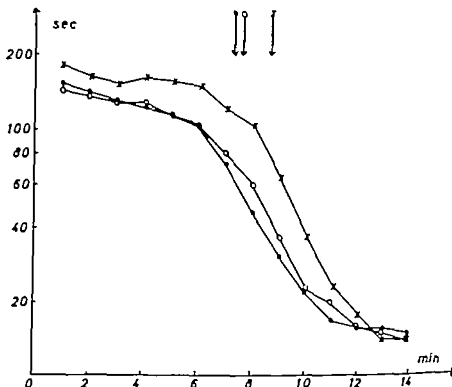


Fig. 3. Thromboplastin activation curve of peripheral blood from 15 normal women immediately after elective Caesarean section was performed.
×—× 14 normal adult women two-stage TAT
●—● 15 normal women immediately after elective Caesarean section, two-stage TAT
○—○ 15 normal women immediately after elective Caesarean section, three-stage TAT

No patient had been in labour prior to the delivery and in all cases the foetus was delivered without any damage to the placenta.

All the women (except for two whose analyses did not differ from the others) had received prophylactic vitamin K, in the form of menadione tablets, 20 mg daily prior to the operation.

Blood samples were also drawn from these women's newborn infants within the first 10 minutes after birth. All the infants were full term and none exhibited any complications while in hospital.

Nielsen, N C Acta obstet. gynec. scand. 48 371 1969 a
Nielsen N C Acta obstet. gynec. scand. 48 392, 1969 b
Schneider C L J Obstet. Gynec Brit. Emp 58 538 1951
Sanderson J L McGowan J J Bunker J P and Goldstein R Anesthesiology 23 92, 1962

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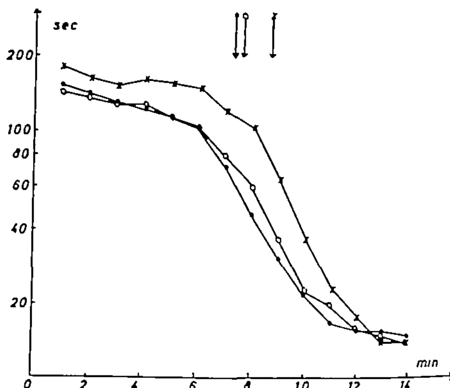


Fig. 3. Thromboplastin activation curve of peripheral blood from 15 normal women immediately after elective Caesarean section was performed.
×—× 14 normal adult women two-stage TAT
●—● 15 normal women immediately after elective Caesarean section, two-stage TAT
○—○ 15 normal women immediately after elective Caesarean section, three-stage TAT

No patient had been in labour prior to the delivery and in all cases the foetus was delivered without any damage to the placenta.

All the women (except for two whose analyses did not differ from the others) had received prophylactic vitamin K, in the form of menadione tablets 20 mg daily prior to the operation.

Blood samples were also drawn from these women's newborn infants within the first 10 minutes after birth. All the infants were full term and none exhibited any complications while in hospital.

The results of *fibrinolysis studies* are also listed in Tables 1 and 2. Comparison of group 1 and 2 shows

A. *Maternal peripheral blood* In the Caesarean section group the fibrinolytic activity in the euglobulin fraction on the standard plates was less than that following vaginal delivery $(0.01 > p > > 0.001)$ but otherwise there were no significant differences between the two groups.

B. *Cord blood*. There was no difference in plasminogen content. On the other hand, fibrinolytic activity measured on the fibrin plates in the Caesarean section group was less marked, all the determinations of fibrinolysis being significantly lower than for the infants delivered by the vaginal route. The p values are thus, for unprecipitated plasma on standard plates $(0.05 > p > > 0.02)$ and on heated plates $(0.01 > p > 0.001)$ and for the euglobulin fraction on standard plates $(0.05 > p > 0.02)$ and on heated plates $(p < 0.001)$

Discussion

In a previous paper (*Nielsen* 1969 b) it was demonstrated that *Caesarean section during labour* has no influence upon the coagulation factors in the maternal or in the cord blood. The fibrinolytic activity is also unchanged in the maternal blood following Caesarean section, while it is reduced in the newborn infants, presumably due to a more gentle manner of birth. In the maternal as well as in the cord blood there were clot promoting components of the same magnitude as following normal vaginal delivery

In the present study it was demonstrated, by the TAT three-stage, that presumably less marked activation of the maternal plasma occurs after *elective Caesarean section* than after vaginal delivery In the plasma of the newborn infants the TAT three-stage clearly demonstrated that no activation of the coagulation process takes place after delivery by elective Caesarean section, unlike the findings following vaginal delivery These results support the theory that during normal delivery there will be activation of the maternal as well as of the infants blood. As mentioned above, this may be due to the passage of tissue thromboplastin and serum to the maternal and foetal circulation during delivery

heated fibrin plates as well as plasminogen, haematocrit, and platelet counts were also done as described previously (*Nielsen* 1969 a).

Results

The results of the *coagulation studies* are given in Tables I and II, and those of the TAT and recalcification times in Figs. 1 2, 3, and 4

Comparison of group 1 (mothers delivered by elective Caesarean section and their infants) and group 2 (normal mothers and their infants) showed

A. *Maternal peripheral blood* The TAT two-stage showed no major differences between the two groups. However t_1 was significantly shortened in the Caesarean section group (0.01 > p > > 0 001) while no significant changes for t_2 t max, or for the recalcification times were demonstrated. In the TAT three-stage there are as described in a previous paper clot-promoting components in the maternal plasma following normal delivery (*Nielsen* 1969 a) In the Caesarean section group the three-stage curve is situated between the 2 two-stage curves except during the first minutes. The recalcification time for the three-stage curve in the Caesarean group is also somewhat longer than the recalcification time for the three-stage curve representing normal mothers.

No other significant differences were found, apart from a reduction in PP (0 01 > p > 0 001) in the Caesarean section group.

B *Cord blood* The recalcification time for infants delivered by Caesarean section was significantly prolonged in the TAT two-stage (0 05 > p > 0 02) and the curve was a little flatter The TAT three-stage showed practically no clot-promoting activity in the plasma of infants delivered by elective Caesarean section, unlike infants delivered by the vaginal route in whom the activity is in the same range as in the maternal blood.

Incidentally there were no changes in coagulation factors in infants delivered by elective Caesarean section apart from a significant reduction in platelet count (0 01 > p > 0 001)

On supplemental examination, confer *Nielsen* (1969 a) this result must be corrected in such a way that the conclusion is that there is no difference of the platelet count for children delivered by elective Caesarean section and for infants delivered by the vaginal route

Nielsen, N C., Acta obstet. gynec. scand. 48 371 1969 a
Nielsen, N C., Acta obstet. gynec. scand. 48, 392, 1969 b
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Vanderveen J L. McGovern J J Bunker J P and *Goldstein R.* Anesthesiology 23 92, 1962

Received on Nov 28, 1969

Incidentally elective Caesarean section does not appear to cause major changes in maternal coagulation factors. The finding that the values for PP (186 per cent) was lower than following vaginal delivery (PP 227 per cent) does not explain, but presumably suggests inaccuracy of the method, the determination being based upon dilution curves plotted on logarithmic paper where the uncertainty is most marked in the high concentrations.

The flatter course of the TAT two-stage and the prolonged recalcification time in cord blood following elective Caesarean section accord with the fact that no activation of the blood took place.

The less pronounced fibrinolytic activity in the maternal blood, and particularly in the cord blood, following elective Caesarean section compared with the findings following vaginal delivery may well be due to the more gentle manner of delivery

That the cyclopropane anaesthesia did not cause changes in coagulation or fibrinolysis factors is in conformity with the studies of *Howland et al* (1959) and *Vanderveen et al* (1962) However the former found increased fibrinolysis in half the patients, but only after major long-lasting operations

SUMMARY

A study of the coagulation and fibrinolysis factors immediately post partum in women delivered by elective Caesarean section and their infants showed compared with normal mothers and their infants

A. Maternal blood Less marked clot-promoting activity in the plasma Otherwise no major changes in the coagulation or fibrinolysis factors.

B Cord blood Prolonged recalcification time and no clot promoting components in the plasma as are invariably present following vaginal delivery

A reduced fibrinolytic activity

REFERENCES

Howland W S Zucker M B Clifton E E and *Boyan C P* Anesthesiology 20 28 1959

Masure R and *Schon e R* Brux-méd 36 1434 1956

Nielsen, N. C., Acta obstet. gynec. scand. 48 371 1969 a
Nielsen, N. C., Acta obstet. gynec. scand. *48* 392, 1969 b
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(77.5 percent) represented treatment with Conluten® (Norethindron 2 mg + Mestranol 0.1 mg). In order to collect as accurate data as possible every woman was given a form on which to note the day of the last withdrawal bleeding and then the two or three first spontaneous menstrual periods. The participants were then asked to mail the form to the author and were informed that when it had been received, a prescription for contraceptive pills for 6 treatment cycles would be available at a drug store. If the form was not received in reasonable time the women were contacted by telephone or by mail. One of the patients could not be traced and was therefore not included in the analysis.

The patients were divided into three groups depending upon their menstrual patterns before the contraceptive therapy. Some of the participants had some difficulty in describing their usual menstrual cycle adequately.

Group I (432 patients) consisted of those who knew or believed that their menstrual cycles had a duration of about one month with a maximum deviation of one week between the shortest and the longest cycles.

Group II (76 patients) consisted of those who had irregular periods.

Group III included those who had regular periods with a duration of about 3 weeks or less or of 5 weeks or more. This group consisted of 7 subjects and was excluded from further analysis.

Thus, the remaining material consisted of 508 patients. Table I shows the number of individuals in the two remaining groups divided into age groups. The mean age in the whole series was 29.9 years.

Results

Figs. 1 and 2 show the distribution of the length of the first three post treatment cycles in Group I and Group II.

Table II gives the mean length of these cycles in the two groups.

Table III shows the percentage of the first three cycles that were less than 36 days and less than 71 days respectively.

Larsson-Cohn, Ulf. Acta obstet. et gynec. scandinav. 48, 416, 1969

From the Department of Obstetrics and Gynaecology (Prof. C. Gemzell)
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THE LENGTH OF THE FIRST THREE MENSTRUAL CYCLES AFTER COMBINED ORAL CONTRACEPTIVE TREATMENT

BY

ULF LARSSON-COHN

Several reports (*Shearman* 1966 and 1968, *Whitelaw et al.*, 1966, *Rice-Wray et al.* 1967) have appeared of persisting amenorrhoea after oral contraceptive therapy. This communication describes the time interval between the last withdrawal bleeding and the first, second and third spontaneous menstrual flow in 516 women who had used combined oral contraceptives for periods between 9 and 55 months.

Material and Methods

The material consisted of 516 women who visited the out-patient clinic of the Department of Obstetrics and Gynaecology at the University Hospital of Uppsala in order to get a renewal of their prescriptions for oral contraceptives. The first 300 patients were recommended to withhold their medication until they had had two spontaneous menstrual periods. In the latter part of the investigation, all new participants were asked to await three menstrual periods before they restarted the therapy. This second group consisted of 216 women.

All patients had used combined drugs only. The duration of the therapy was between 9 and 55 months with a mean of 22.5 months. Most combined oral contraceptive drugs available on the Swedish market had been used but the majority of the cycles

Table I. *Number of Women in Group I (Regular Periods) and Group II (Irregular periods) Divided into Age Groups*

Age in Years	Group I	Group II
17–20	20	1
21–30	253	52
31–40	140	21
41–49	19	2

Table II. *Mean Duration in Days of the First Three Post-Treatment Cycles in Group I (Regular Periods) and Group II (Irregular Periods)*

	Group I	Group II
First post-treatment cycle	36.0	41.0
Second post-treatment cycle	29.9	36.8
Third post-treatment cycle	29.6	37.3

Table III. *Percentage of the First Three Post-Treatment Cycles that Were Less than 36 and 71 Days, Respectively*

		Group I	Group II
First post-treatment cycle	$\leq$35 days	50.3	33.4
	$\leq$70	98.2	94.9
Second post-treatment cycle	$\leq$35 days	88.6	51.3
	$\leq$70	99.7	93.3
Third post-treatment cycle	$\leq$35 days	88.0	48.9
	$\leq$70	99.5	88.5

Seventeen pregnancies occurred during the observation time, 2 of these during the first post-treatment cycle and 12 during the second. Seven of these subjects declared that they had tried to get pregnant and 7 that they had tried to avoid pregnancy. It was not possible to get a direct answer in 3 cases.

Four patients, one of which belonged to Group 1 and three

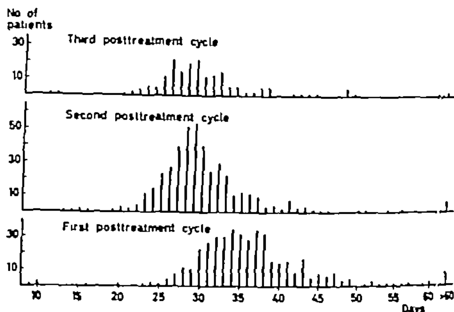


Fig. 1 Duration of the first three post treatment cycles among the patients who had regular periods before treatment.

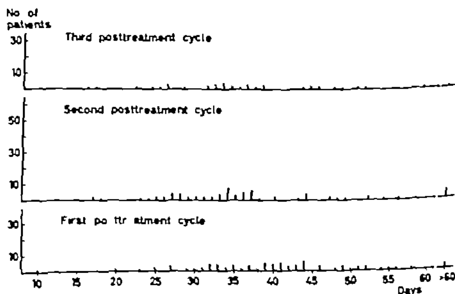


Fig 2. Duration of the first three post treatment cycles among the patients who had irregular periods before treatment

There is reason to believe that the same variability existed in the present series, a circumstance that must be kept in mind when the results are discussed. This fact, however does not exclude the validity of dividing the material into the two groups described earlier It is reasonable to assume that those patients who declared that their cycles usually had a length of about 28 days differed statistically from those who said that their periods used to be irregular

This study shows that in most subjects who usually have regular periods, the first menstrual cycle after combined oral contraceptive therapy is longer than the following ones. The mean difference is about 6 days with a considerable variation in both directions.

Bell and *Loraine* (1967) studied 10 subjects during the first and second cycle after combined oral contraceptive therapy Their conclusion was that the variations from normality in respect of steroid and gonadotrophin output were relatively minor and mainly confined to the first post-treatment cycle.

This investigation shows that in women with regular periods the pre treatment menstrual pattern is usually restored by the second post treatment cycle a fact that might be explained by the study of Bell and *Loraine Rice-Wray et al.* (1967) studied a group of 125 women who had used combined drugs for up to 76 cycles. They found that in 2 of their cases the first post-treat ment cycle was longer than 180 days. However no information was given about the menstrual patterns before the therapy

In the present study 4 out of 515 subjects developed amenor rhoea lasting more than 180 days. It is noteworthy that 3 of these 4 women had had irregular menstrual periods before the therapy The main conclusion of this investigation is therefore that combined oral contraceptive agents should be used with caution in women who have irregular menstruation especially in those who have not completed their family

Rice Wray et al also studied a group of 38 women who had used sequential regimes and found a higher percentage of first post treatment cycles of normal length (24–35 days) than among those who had used combined drugs. As the series was very limited it is doubtful if any valid conclusion may be drawn from

who belonged to Group 2 did not get their first spontaneous menstrual bleeding within 6 months. They are here described in detail

Case 1 Unmarried university student 26 years old. Before therapy periods always regular with a duration of about 28 days. An illegal abortion at 23 years of age. Took contraceptive tablets during 26 cycles after which she had an amenorrhoea of 9 months duration. She was then referred to the hospital for a complete endocrinological investigation. Biopsy showed a proliferative endometrium. There was a relatively low excretion of FSH and LH. After stimulation with human FSH she showed with a marked increase of total oestrogens in the urine. The case was interpreted as a persisting hypothalamic pituitary depression.

Case 2 Unmarried 24 years old university student who had never been pregnant. Always irregular bleeding with a cycle length usually between 4 and 7 weeks. Had taken contraceptive tablets for 24 cycles. The first spontaneous menstrual period which was described as fairly normal, occurred 4 months after her last withdrawal bleeding. She had no further menstrual period during the following 5 months. After that time she left Sweden and was lost for follow-up.

Case 3 Pharmacist, unmarried 23 years old. Had never been pregnant. Always irregular cycle with a duration usually between 1 and 2 months. Oral contraceptives for 2 years. After that amenorrhoea for 11 months, because of which she was scheduled for an endocrinological investigation. She was however in the meantime prescribed gestagenic hormones cyclically by another gynaecologist As these hormones gave her regular bleeding she refused to be admitted for investigation.

Case 4 Married 29 years old with 2 children and one therapeutic abortion. Irregular menstruation after the birth of the second child. This tendency was more pronounced after the abortion. She was prescribed oral contraceptives after amenorrhoea of more than one year's duration and took the tablets for 2 years. The first post treatment cycle had a duration of 39 days after which she developed amenorrhoea that still persisted 10 months later She is scheduled for an endocrinological investigation.

Discussion

Treolar et al (1967) and *Chiaz e et al* (1968) showed in their large prospective studies that in their series the majority of women had a considerable variation in the length of their menstrual cycles although most of them thought that their periods were regular In the age group 25–34 years for example 20 per cent of the cycles had a duration of less than 25 days or more than 32 days.

Nieminen, Usko, von Numers, Claes and Purola, Esko, Acta obst. et gynec. scandinav. 48, 423, 1969
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PRIMARY SARCOMA OF THE OVARY

BY

USKO NIEMINEN, CLAES VON NUMERS AND ESKO PUROLA

Primary sarcoma of the ovary is one of the least common gynaecological neoplasms. *Miller* (1937) reviewed 25 series comprising a total of 12,092 ovarian tumours, of which 343 were ovarian sarcomas (2.84 per cent). In a series of 231 ovarian tumours *Vrafolu* and *Manolescu* (1964) detected only three sarcomas (1.3 per cent). According to *Novak* and *Woodruff* (1967) the ratio of sarcoma of the ovary as compared to carcinoma is about one to 40. Both *Haines* and *Taylor* (1962) and *Vrefolu* and *Manolescu* maintain that in those series reporting an exceptionally high ratio of sarcoma, other types of tumour—e.g. thecoma, granulosa cell tumour, dysgerminoma, arrhenoblastoma—have been erroneously diagnosed as sarcoma. In addition, *Haines* and *Taylor* point out that sarcomatous areas may be predominant in a malignant teratoma, and that oedematous, haemorrhagic or degenerate fibromas may be confused with sarcoma.

Ovarian sarcoma is encountered in all age groups, but in contrast to other malignant tumours it has been found in exceptionally young females. In *Miller*'s survey the age groups 21–30 and 41–50 predominated, but 15 per cent of the patients were aged 11–20 years and about 4 per cent were under 10 years old. Occasional cases of sarcoma in foetuses and infants have been reported.

Ovarian sarcoma may be bilateral and this was found in 24.9 per cent cases in Miller's review.

On the basis of the microscopic structure, ovarian sarcoma may

their investigation In the absence of more reliable data, it is nevertheless possible that the sequential regime should be preferred when oral contraceptives are prescribed for women with irregular menstrual periods especially those who have not completed their childbearing period.

SUMMARY

The length of the first three menstrual cycles after combined oral contraceptive therapy was investigated in 516 women. The material was divided into those who had regular (Group I) and those who had irregular bleeding (Group II) before the therapy

In Group I the first post treatment cycle was about 6 days longer than the following ones which had about the same duration as before the therapy Four patients developed amenorrhoea that persisted for more than 6 months Three of these patients belonged to Group II It is recommended that combined oral contraceptives should be prescribed with caution to women with irregular menstrual periods who have not completed their childbearing period.

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PRIMARY SARCOMA OF THE OVARY

BY

USKO NIEMINEN, CLAES VON NUMERS AND ESKO PUROLA

Primary sarcoma of the ovary is one of the least common gynaecological neoplasms. *Miller* (1937) reviewed 25 series comprising a total of 12,092 ovarian tumours, of which 343 were ovarian sarcomas (2.84 per cent). In a series of 231 ovarian tumours *Vrejotu* and *Manolescu* (1964) detected only three sarcomas (1.3 per cent). According to *Novak* and *Woodruff* (1967) the ratio of sarcoma of the ovary as compared to carcinoma is about one to 40. Both *Haines* and *Taylor* (1962) and *Vrejotu* and *Manolescu* maintain that in those series reporting an exceptionally high ratio of sarcoma, other types of tumour—e.g. thecoma, granulosa cell tumour, dysgerminoma, arrhenoblastoma—have been erroneously diagnosed as sarcoma. In addition, *Haines* and *Taylor* point out that sarcomatous areas may be predominant in a malignant teratoma, and that oedematous, haemorrhagic or degenerate fibromas may be confused with sarcoma.

Ovarian sarcoma is encountered in all age groups, but in contrast to other malignant tumours it has been found in exceptionally young females. In Miller's survey the age groups 21–30 and 41–50 predominated, but 15 per cent of the patients were aged 11–20 years and about 4 per cent were under 10 years old. Occasional cases of sarcoma in foetuses and infants have been reported.

Ovarian sarcoma may be bilateral and this was found in 24.9 per cent of cases in Miller's review.

On the basis of the microscopic structure, ovarian sarcoma may



Fig. 1 Case 1 Reticular tumour tissue rich in cells in structure resembling endometriotic stroma tissue
Haemat. + van Gieson × 240

be classified in various ways The nomenclature is noticeable inconsistent and varied. *Miller* suggested a division into anaplastic or poorly differentiated tumours on the one hand and well differentiated neoplasms on the other In the former group he included round cell spindle cell and giant cell tumours, and also melanosarcomas in the latter fibroblastic lipoblastic, chondroblastic osteoblastic and myoblastic tumours and adenosarcomas. In addition, *Baldas* and *Lászlo* (1965) cited descriptions of ovarian tumours classified as leiomyo- rhabdomyo- carcino- and neurofibrosarcoma, and *Novak* and *Woodruff* mentioned angiosarcoma. It seems possible that the tumours in question were teratomatous.

According to *Novak* and *Woodruff* an ovarian sarcomatous tumour is generally macroscopically moderate in size and as a rule lobulated or nodular The cut surface is firm in some places but there is invariably a tendency towards necrosis with the production of soft pultaceous areas or ragged cystic cavities.

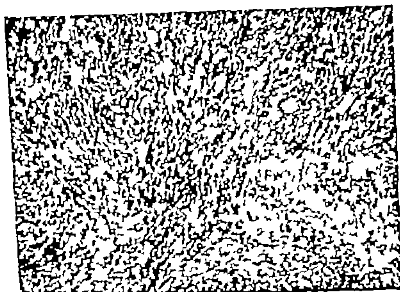


Fig. 2. Case 2. Slightly differentiated fibrosarcoma. Haemat. an Gieson ×120.

Material

During the years 1945–1966 a total of about 2800 patients with benign ovarian neoplasms, and 1090 with malignant primary ovarian neoplasms were treated at Departments I and II of Obstetrics and Gynaecology, Helsinki University Central Hospital. Five of these tumours, detailed in this paper, were ovarian sarcomas. The frequency was thus 0.16 per cent of the total series and 0.46 per cent of the malignant cases. Originally a diagnosis of ovarian sarcoma was made in 16 cases, but some of these were secondary tumours and in others the diagnosis was altered on re-evaluation of the specimens.

Case 1. A 55-year-old unmarried nulliparous woman had left oophorectomy performed on August 18, 1950. The uterus and the right adnexa were normal. The left ovary was replaced by a tumour about 8 cm in diameter, smoothly rounded in outline, which was extensively adherent to the sigmoid colon. — Pathologist's report: The specimen shows solid tumour tissue forming exten-

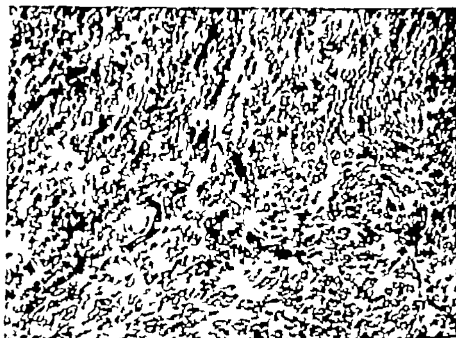


Fig. 3 Case 2. Same tumour tissue as in Fig. 2.
Haemat. + van Gieson. × 240

are irregularly shaped areas. Between these without any sharp demarcation from the tumour tissue there is stroma composed of connective tissue poor in cells. The tumour tissue is highly cellular slightly polymorphocellular and in parts clearly reticular in structure. Mitoses are scanty. The structure of the tumour undoubtedly justifies a diagnosis of sarcoma of low grade malignancy originating in stromal endometriosis. (Fig. 1) —Postoperatively the patient was given radium and radiation therapy. She was last seen on March, 1959. No recurrence or changes indicative of metastases were then observed. The patient died on Sept. 10, 1960 from cardiac infarction and diabetes.

Case 2. A 48-year-old nulliparous married woman had bilateral salpingo-oophorectomy performed on Nov. 11 1952. In addition, resection of the ileum and bladder was carried out. From the left ovary a tumour extended to two finger-breadths below the level of the umbilicus. It ruptured on removal, and a greenish thin fluid containing necrotic pieces of tissue flowed out.—*Pathologist's report.* The tumour tissue shows infiltrative growth and is highly cellular. The cells are relatively small and their nuclei are slender or rounded. Occasional fibres occur in the tumour tissue. Mitoses are found in moderate numbers. The tumour is a slightly differentiated fibrosarcoma. (Figs. 2 and 3) —Postoperatively the patient received radiation therapy. She died from extension of the ovarian tumour on Feb. 19 1954.

Case 3. A 50-year-old married woman had four children and no abortions.



Fig 4 Case 3 Polymorphocellular fibrosarcomatous tissue
Haemat + van Gieson × 240

Subtotal hysterectomy and bilateral salpingo-oophorectomy were carried out on Sept 3, 1955 There was nodular encapsulated tumour about 7 cm in diameter arising from the left ovary The cut surface of the tumour was pale and homogeneous —Pathologic port The specimen shows highly cellular tumour tissue of mesenchymal type In parts the nuclei are rather long and slender in parts rounded, polymorphous, arying in chromatin concentration Mitoses are seen in abundance By means of lipoid staining, which was nega-tiv the possibility of theca-cell tumour is eliminated The tumour is fibrosarcoma, ariable in degree of differentiation (Fig. 4) Postoperatively the patient was given radiation therapy She died from her ovarian tumour on Oc 6, 1964

Case 4 A 70-year-old married woman had four childre and no abortions Subtotal hysterectomy and bilateral salpingo-oophorectomy were carried out on May 4, 1959 In addition, appendicectomy was performed From the left ovary fluctuant tumour with dark surface and smooth rounded outline extended 5 cm above the umbilicus The tumour weighed 5570 g When bisected it was found to be multilocular cyst, containing old blood —Pathologic report In specimens from those parts of the tumour wall which contains relatively small cysts, cavities of arying size occur lined by simple columnar or cuboidal epithelium In the non-cystic parts the tumour wall consists of two different layers of tissue an outer layer made up of dense

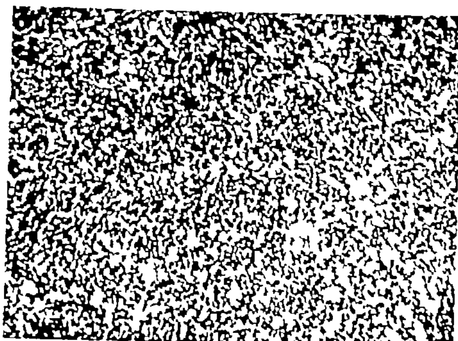


Fig. 5. Case 4 Highly cellular tumour tissue, composed of atypical smooth muscle tissue
Haemat. + van Gieson. × 120

connective tissue and an inner layer in some places of considerable thickness, made up of relatively closely packed and moderately pleomorphic cells. The cells are either fusiform in shape or more or less stellate with numerous anastomosing protoplasmic processes. Mitoses occur in relative abundance. The tissue is undoubtedly atypical smooth muscle tissue The microscopic examination suggests a diagnosis of serous ovarian cystadenoma with benign epithelial components and atypical and obviously malignant smooth muscle tissue infiltrating the wall. (Figs. 5 and 6) Postoperatively the patient received radiation therapy She was still alive on Jan. 12, 1966.

Case 5 A 62 year-old married woman with no data a ailable regarding parity underwent exploratory laparotomy on Jan. 29 1963. The lower part of the abdomen was filled by an inoperable tumour some 20 cm in diameter When removal was attempted the tumour ruptured and serous fluid flowed out. The tumour was in part cystic.—*Pathologist s eport* The specimen shows richly vascularized polymorphocellular tumour tissue clearly reticular in structure. The tumour cells anastomose by plasma processes some of which are noticeably long The nuclei show a wide variation in size and shape. Multinucleated giant cells occur Mitoses are abundant. The reticular structure seems to be indicative of a tumour originating in endometriotic stroma tissue.



Fig. 6 Case 4 Same tumour tissue as in Fig. 5.
Haemat van Gieson ×240

However owing to the marked atypia and polymorphism this assumption remains open to dou t (Figs. 7 and 8) —Postoperatively the patient received radiation therapy She died on May 9 1963, from ovarian tumour

Discussion

Primary ovarian sarcoma is indeed a rare tumour as is borne out by the incidence found in this series. This supports the impression that report indicating an exceptionally high frequency arise from errors in interpretation of the specimens. In our hospital a total of 16 ovarian sarcomas were diagnosed in 20 years. After thorough perusal of the hospital records and re-evaluation of the histological specimens only five cases proved to be primary ovarian sar oma

A compared t previous reports the mean age of our patients (57 years) was relatively high The youngest patient was 48 and the oldest 0 All cases were diagnosed after the menopause.

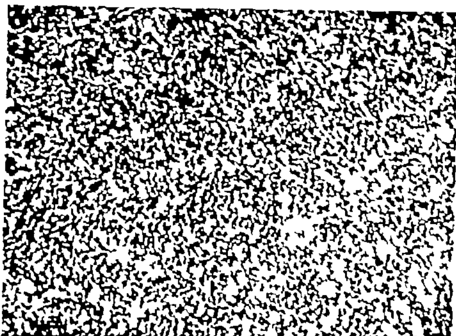


Fig. 5. Case 4. Highly cellular tumour tissue composed of atypical smooth muscle tissue.
Haemat. + van Gieson. × 150.

connective tissue and an inner layer, in some places of considerable thickness, made up of relatively closely packed and moderately pleomorphic cells. The cells are either fusiform in shape or more or less stellate with numerous anastomosing protoplasmic processes. Mitoses occur in relative abundance. The tissue is undoubtedly atypical smooth muscle tissue. The microscopic examination suggests a diagnosis of serous ovarian cystadenoma with benign epithelial components and atypical and obviously malignant smooth muscle tissue infiltrating the wall. (Figs. 5 and 6). Postoperatively the patient received radiation therapy. She was still alive on Jan. 1, 1966.

Case 5. A 6_-year-old married woman with no data available regarding parity underwent exploratory laparotomy on Jan. 29, 1963. The lower part of the abdomen was filled by an inoperable tumour some 20 cm in diameter. When removal was attempted the tumour ruptured and serous fluid flowed out. The tumour was in part cystic.—*Pathologist's report.* The specimen shows richly vascularized polymorphocellular tumour tissue, clearly reticular in structure. The tumour cells anastomose by plasma processes, some of which are noticeably long. The nuclei show a wide variation in size and shape. Multinucleated giant cells occur. Mitoses are abundant. The reticular structure seems to be indicative of a tumour originating in endometriotic stroma tissue.

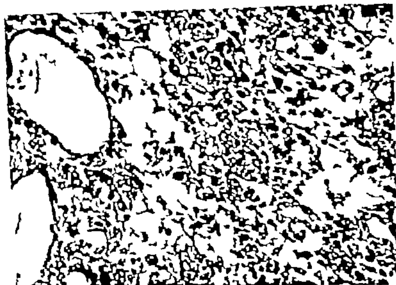


Fig. 8 Case 5 Same tumour tissue as in Fig. 7
Haemat. ... van Gieson 240

Baldzs and *László* (1965) ha e each described one case of leiomyosarcoma. *Istre* (1951) described four cases, in three of which the presence of myogenic elements was an outstanding histological feature. In contrast to this type of myosarcoma, rhabdomyosarcoma has been reported several times.

In one case (no. 1) the sarcoma obviously originated in ovarian endometriosis, and in a further case (no. 5) the genesis was probably the same. *Benjamin* and *Campbell* (1960) described one such case and mentioned that they had not seen any previous similar report. Sarcoma originating in endometriotic tissue in other parts of the genital tract, the uterus in particular, is a more familiar phenomenon. About 100 such cases of stromal endometriosis ha been reported in the English literature (*Stearns*, 1958). Stromal endometriosis, also called stromatous endometriosis *stromatosis and stromal adenomyosis*, has been recognized and described as an entity for many years, but its histogenesis, pr gnosis and possible r lationship with endometriosis and endomet ial sarc ma are not yet fully understood.

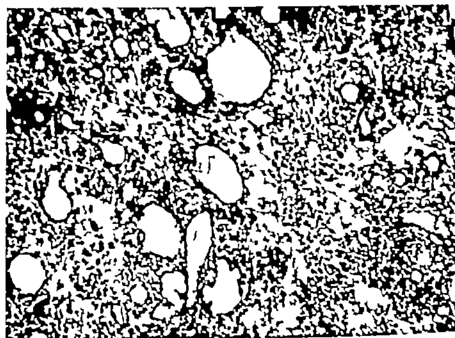


Fig 7 Case 5 Reticular polymorphocellular sarcomatous tissue. Haemat. + van Gieson. × 100

However as children are not admitted to our hospital this age group remains outside the scope of this study

All the present cases were unilateral All tumours were relatively large The smallest one was about 7 cm in diameter and the largest filled almost the whole of the abdominal cavity

Three of the patients have subsequently died from ovarian sarcoma. One died from cardiac infarction ten years after operation and one is still alive

In two cases (nos. 2 and 3) the tumour was a fibrosarcoma, which is not considered as an unusual type of ovarian sarcoma. Giant follicular lymphoid hyperplasia morbus Brill Symmers, (*Schröter* 1962) sometimes develops in association with ovarian fibrosarcoma, with enlargement of lymph nodes in various parts of the body Glandular enlargement was not observed in our two cases

In one case (no 4) the structure of the tumour was that of leiomyosarcoma This case has previously been described by *von Numers* and *Mikkonen* (1960) *Kelley* and *Scully* (1961) and

Allander Erik, and Wågermark, Jan, Acta obst. et gynec. scandinav. 48, 433, 1969

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LEYDIG CELL TUMOURS OF THE OVARY

Report of Three Cases

BY

ERIK ALLANDER AND JAN WÅGERMARK

Leydig cell tumour of the ovary is a rare usually virilizing tumour Other ovarian and adrenal tumours can also be virilizing and the main problem for the clinician is to localize the source of the excessive hormone production. The morphologist on the other hand, has to face the fact that histologically identical tumours may differ considerably in their endocrine activity All these facts have caused confusion, and a number of partially or completely synonymous terms have appeared in literature

On the basis of three cases the clinical and morphological problems will be discussed with emphasis on ontogenetic and biochemical aspects

Case I Woman born in 1903 No family history of diabetes. II-grav II-para, menarche at the age of 14 and menopause at the age of 52 (1955). Hypertension and angina pectoris since 1960 In 1959 hypertrichosis was noticed on fac and neck When admitted t hospital in 1963 the patient had a male voice obvious facial hypertrichosis and hypertrophy of the clitoris Distribution of pubic hair was normal The appearance was not typical of Cushing' syndrome

Laboratory tests showed normal glucose tolerance and repeatedly normal values for urinary 17 ketosteroids (17-KS) and 17-ketogenic steroids (17 KGS) (5–7 mg and 16–18 mg/24 h) No reduction of 17-KS in dexamethasone suppression test (8 mg) but normal reduction of 17-KGS An ovarian tumour

Reprints from E Allander Department of Social Medicine Karolinska Institutet, 104 01 Stockholm 60, Sweden

SUMMARY

The literature on ovarian sarcoma is reviewed. Five primary ovarian sarcomas are described which were discovered in a series of 3890 operatively treated ovarian tumours. On the basis of their histological structure two were classified as fibrosarcomas. In one case the neoplasm was a very rare mixed tumour with leiomyosarcomatous tissue elements in the wall of a benign serous cystadenoma Sarcoma originating in an ovarian endometriotic focus was obviously present in one case and probably in another Only one similar case has previously been described.

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Case 1 Woman born in 1903 No family history of diabetes. II-grav II-para, menarche at the age of 14 and menopause at the age of 52 (1955). Hypertension and angina pectoris since 1960 In 1959 hypertrichosis was noticed on face and neck When admitted to hospital in 1963 the patient had male [illegible] obvious facial hypertrichosis and hypertrophy of the clitoris Distribution of pubic hair was normal The appearance was not typical of Cushing's syndrome

Laboratory tests showed normal [illegible] glucose tolerance and repeatedly normal values for urinary 17 ketosteroids (17-KS) and 17-ketogenic steroids (17 KGS) ([illegible] mg and 10–18 mg 24 h) No reduction of 17 KS in dexamethasone suppression test (8 mg) but normal reduction of 17 KGS An ovarian tumour

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was considered most probable and at operation the right ovary was found to be somewhat enlarged. It contained a well-defined tumour with a diameter of 1.5 cm situated in the hilar region.

Microscopically the tumour consisted of cells with abundant eosinophilic cytoplasm, several containing so called Reinke crystalloids. The nuclei were distinctly outlined, had a loose network of chromatin and often a prominent nucleolus. The cells were closely packed without any special arrangement. Diagnosis Leydig cell tumour

In January 1967—four years after the operation—the virilization had regressed but the patient still had moderate facial hypertrichosis. Her voice was now normal and the clitoris of ordinary size. Laboratory values for 17 KS and 17 KGS were normal (4.3 and 14.4 mg/24 h)

Case II Woman born in 1897 *Diabetic mother 0-grav.*, menopause at the age of 49 (1946) Diabetes since 1954 well controlled with oral hypoglycaemic agents (chlorpropamide) In 1958 the patient began to become bald and facial hypertrichosis appeared. On her legs and back the growth of hair increased When admitted to hospital in 1960 she was practically bald but had facial hypertrichosis. Normal pubic hair and no hypertrophy of the clitoris or Cushingoid appearance There were normal values for urinary 17 KS and 17 KGS (5–9 mg and 16–18 mg/24 h) On dexamethasone suppression test (8 mg) no reduction of 17 KS on the other hand a normal reduction of 17 KGS

An ovarian tumour was suspected clinically and a bilateral oophorectomy was performed. The left ovary contained a tumour with a diameter of 1 cm in the hilar region. The right ovary contained a somewhat larger cystic tumour Histological examination showed that the tumour from the left ovary was made up of polygonal cells with fairly large pale nuclei with distinct nucleoli The cytoplasm contained lipochrome pigment and Reinke crystalloids In the surrounding tissue there were some islands of hyperplastic hilar cells. The wall of the cyst tumour in the right ovary consisted of similar tumour tissue Neither of the two tumours showed any signs of malignancy Diagnosis Bilateral Leydig cell tumour

Two years after the operation the scalp hair had begun to return, and the facial hypertrichosis decreased Values for urinary 17 KS 17 KGS oestrogens and gonadotrophines were normal for her age

Case III Woman born in 1903 No family history of diabetes. 0-grav menarche at the age of 14 and menopause at the age of 50 (1953) She has diabetes since 1965 and has been treated with insulin In 1958 baldness facial hypertrichosis and deepening of the voice were noticed. In 1966 when she was admitted to hospital an increased growth of hair on her shoulders and back could also be noticed as well as a growth of pubic hair spreading up on the abdomen Moderate hypertrophy of the clitoris but not Cushingoid appearance

Laboratory tests showed normal values for cortisol in plasma, increased 17 KS and 17 KGS (16–36 mg and 15–28 mg 24 h) Tetrahydro E and tetrahydro F were 1.8 and 1.5 mg/24 hr respectively Urinary pregnantriol was

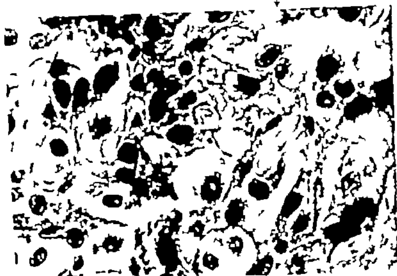


Fig 1 Tumour tissue composed of cells rich in pale, slightly eosinophilic cytoplasm Note the big Reinke crystalloid in the right half of the picture 500 Case III

2.9 mg/24 h (upper borderline value) N suppression of 17 KS on Dexamethasone test (8 mg) nor of 17 KGS This rose the suspicion of an adrenal cortical tumour

Radiographic examinations (aortography cochaecography and pelvic arteriography) did not show any changes in the adrenals or in the region of the ovary Transient anuria appeared after these arteriographies which were performed simultaneously

A bilateral exploration of the adrenals wa performed and on the right side pea-sized tumour-like formation was found and removed The left adrenal was normal Microscopical examination of the right adrenal showed active tuberculosis No signs of TB in other organs could be found despite careful bacterial and radiological examinations

The ason for the virilization then had to be an ovarian tumour and a urological laparatomy was made The lower part of the abdominal cavity was filled with mucus originating from benign ruptured mucocoele of appendix The lef ary was apparently normal The right ovary which was removed, contained tumour with diameter of 1.5 cm Microscopically the tumour was similar those of the other two patients and contained lipofuscin pigment and Reinke crystalloids (Fig 1) There were no signs of malignancy The diagnosis was Leydig ll tumour

Discussion

Ovarian Leydig cell tumours are said to be rare and few cases have been published As recently as 1959 there were only about 10 cases to be found in the literature (*Merrill* 1959) By 1966 the number had increased to 50 (*Dunnihoo et al* 1966) However we have found reports of another two cases (*Halinska et al.* 1964 *Marsiglia et al* 1965) we know about three which are being prepared for publication (*Ernest* personal communication) and have seen three ourselves. Evidently this tumour is not as uncommon as is generally believed.

Morphology For the morphologist the problem is to distinguish between tumours composed exclusively of closely packed "lipid cells This group consists of Leydig cell tumours adrenal rest tumours and the folliculome lipidique (Lecène) (see *Morris et al* 1958) It is almost impossible to differentiate between these types. Only the tumours containing cells with the cytoplasmic bodies called Reinke crystalloids can be recognized as Leydig cell tumours The presence of these crystalloids in Leydig cells is not usual In normal testicular tissue they do not appear until after puberty and then are visible in only about 6 per cent of the Leydig cells (*Gardener et al* 1957) With the electron microscope however it has been possible to demonstrate cytoplasmic structures of submicroscopical size which probably represent earlier phases in the formation of Reinke crystalloids.

Function If the morphology is similar within the group of lipid cell tumours the opposite is true of their function. The Leydig cell tumours for example are usually virilizing but cases with primarily oestrogenic effects have been described in the survey of *Dunnihoo et al* (1966) This is easily understood since the intracellular synthesis of both androgens and oestrogens starts with progesterone It has to be remembered that this substance is also the precursor of corticoids The different pathways and their interrelationships for the synthesis of steroid hormones are well known from *in vitro* investigations (*Besch et al* 1964 *Kase et al* 1964 *Lipsett et al* 1966 *Samuels et al* 1967 *Sandberg et al* 1962 *Savard et al* 1961 *Steinberger et al* 1967) The cells of the "lipid cell tumours are potentially producers of steroid

hormones, and their enzyme complement determines which phases of the synthetic pathway will be dominant—and thereby also the clinical effects virilization, feminization or Cushing syndrome (*Gabrilove* 1964)

Ontogeny The occurrence of adrenal (or adrenal rest) tumours in the ovaries is easily explained by the ontogenetic development of the gonads. These consist of three components and develop from the genital ridge, a thickening of the rim of the mesonephros. Their *first component* consists of a primitive mesenchyme. It is possible that the granulosa cells have their origin in this part. The *surface of the ridge* is covered by the second component, coelomic epithelium, from which the adrenal cortex is formed. The primordial sex cells, which constitute the third component, migrate to the area, probably from the intestinal region (see *Morris et al.* 1958)

The Leydig cells of the testicle as well as the so-called ovarian hilar cells differentiate from the primitive mesenchyme These cells are normally to be found in the hilar region at the ovary in about 80 per cent of post-pubertal women (*Gardener et al.* 1957 *Sternberg*, 1949) and they constitute the origin of the Leydig cell tumours. It is worth noting that these tumours often identical in structures and function, can be of mesenchymal as well as of epithelial origin

Suggestive Diagnostic Nomenclature Our discussion has shown that it has to be difficult or impossible to distinguish morphologically between ovarian tumours composed solely of "lipid cells In this we agree with a recently published paper by *Taylor* and *Norris* (1967) We believe that it would be both adequate and sufficient to present the morphological diagnosis as potentially steroid producing ovarian tumour in addition to a judgement of the grade of malignancy based on the usual criteria. To that description available data on steroid production can be added, (e.g. benign potentially steroid-producing ovarian tumour testosterone-producing) According to current nomenclature of pathology (SNOP) (*College of Amer Pathologists* 1965) there is a possibility of making a diagnosis to different levels of precision using these principles.

Clinical Diagnosis. Since ovarian Leydig cell tumours practi-

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cally always are small they will be diagnosed only when they produce hormones usually androgens The clinical diagnosis in a female patient with virilism will be either ovarian or adrenal disease. It is well known that high as well as low values for the excretion of 17 KS and 17 KGS are seen in both ovarian and adrenal tumours This is in accordance with the previous discussion which shows that biochemical analysis of the hormone production will give no information on the location of the tumour Analysis of the steroid pattern in blood from the venous side of the respective organ might be tried (*Saez et al* 1967)

Angiographic diagnosis is difficult and was not successful in our three cases. Retroperitoneal air insufflation is of limited value in revealing adrenal tumours.

In conclusion then it is clinically often impossible to differentiate between adrenal and ovarian virilizing "lipid cell tumours, and a surgical exploration has to be performed. An analysis of blood testosterone levels and metabolism may be useful.

It is noticeable that among the 55 cases of ovarian Leydig cell tumours published up to now (our cases included) eleven were diabetics. The frequency of diabetes mellitus however is high in this age group but a connection between the two conditions might exist.

SUMMARY

Three cases of virilizing Leydig cell tumours of the ovary are presented, and some of the diagnostic problems are discussed in connection with morphogenesis morphology steroid synthesis and the clinical picture Difficulties appear when distinguishing morphologically between Leydig cell tumours and other lipid cell ovarian tumours and in differentiating clinically an ovarian tumour from one in the adrenal glands

On the basis of data from morphology function ontogeny and clinic it is suggested that the description of the steroid producing group of ovarian tumours should be simplified according to current nomenclature (SNOP)

Table I. *Reports on Cure Rates with Metronidazole*

Author	Year	Patients	Cure Rate
[illegible] et al.	1961	108	96 %
Arnold (combined therapy)	1961	140	89 %
Keighley	1962	102	100 %
Svendsen	1962	76	97 %
Bredland	1962	82	98 %
Csonka	1963	93	92 %
Teton and Treadwell	1963	2168 (summary of 48 studies)	98 %
Scott-Gray	1964	300 (pregnant women)	95 %
Gardner and Dukes	1964	244	97 %
Pereyra and Dee Lansing	1964	2002	98 %
Perl	1965	151 (pregnant women)	100 %
Parker et al.	1965	420	97 %
Peterson et al	1966	206 (pregnant women)	99 %
Sands	1966	123 (pregnant women)	98 %
Arnold (combined therapy)	1966	72	76 %

Table II *Age Distribution*

15–19 years	13 patients
20–29 years	48 patients
30–39 years	28 patients
40–49 years	17 patients
50–59 years	14 patients
Total	120 patients

Table III *Treatment Results with Metronidazole*

	Patients	Not Cured	Cured	Cured after 2 Courses
Course I	120	45	73 (67 %)	
Course II	45	29	15 (34 %)	88 (73 %)

Two patients did not return for examination after course I
One patient did not return for examination after course II

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METRONIDAZOLE TREATMENT OF TRICHOMONAL VAGINITIS

A Comparison of Cure Rates in 1961 and 1967

J CHR. AURE AND H. GJØNNÆSS

Introduction

Until metronidazole was introduced as a trichomonacide, the results of treatment of vaginal trichomoniasis were poor (*Willcox* 1961) In 1958 the trichomonacidal effect of metronidazole was found to be excellent in both *in vivo* and *in vitro* experiments (*Cosar* and *Joulou* 1959) and so were the results of the first reported clinical trial (*Durel et al* 1959) Since then several authors have reported cure rates of 90–100 per cent (Table 1)

In 1962 *Svendsen* from this department reported a cure rate of 97 per cent (74/76) In our daily routine during recent years we have had the impression that the therapeutic effect of the drug is not as good as previously We have therefore performed a new clinical trial with metronidazole

Material and Method

The series consists of 120 patients with trichomonal vaginitis seen in the gynaecological out patient clinic at Lillehammer Hospital from February 1967 to December 1967 They were selected according to *Svendsen* (1962) The diagnosis of trichomoniasis was verified by microscopic examination of wet smear preparations stained with 0 25 per cent. Brilliant Cresyl Blue in isotonic saline. Cervical smears for gonococcal cultures were taken in all patients and were positive in 9 cases

All patients were given 200 mg metronidazole orally three

agreement between cytological and wet smear preparations. Failure to diagnose the presence of flagellates before treatment would exclude the patient from our trial. If the same occurred at the follow-up examination, the cure rate would improve. Thus diagnostic failures cannot explain our bad treatment results

In comparing our treatment results with those of *Svendsen* (1962) some differences in the composition of the series should be noted. *Svendsen* excluded all pregnant women, and none of his patients had received metronidazole treatment before. In his sample 46 out of 76 patients had established regular sexual partnerships (61 per cent) in our series the percentage was 63 Except for these slight differences the series should be as similar to each other as possible—coming from the same population and treated in the same department. The treatment was identical in both series except that none of Svendsen's cases received more than one course At examination during the first postmenstrual period after treatment Svendsen found only two failures and at a second follow-up 3 months later there were 7 with reinfestation.

The bad results of treatment in pregnant women cannot explain the difference If these patients are excluded from our series the cure rate after one course of treatment would be 64 per cent (65/102) and after two courses 78 per cent (79/102) Both of these differences from Svendsen's 97 per cent are statistically highly significant ($p < 0.0005$)

Reinfections are probably not more frequent in 1967 than in 1961 the percentage of fixed sexual partnerships being the same

Jennison et al. (1961) and *Scott-Gray* (1964) found no evidence of reduced absorption of the drug in those cases which did not respond to treatment. The manufacturers of the drug have informed us that no changes in the composition of the tablets have been made during these years

Treatment with higher doses or combined oral and vaginal treatment do not seem to increase the cure rates (*Pereyra* and *Dee Lancing*, 1964 *Watt* 1965 *Sands* 1966)

Already in 1962 *Watt* and *Jennison* concluded that development of resistance against metronidazole was a theoretical pos-

times a day for seven days. The male partners were treated simultaneously when possible If the first course of therapy failed a second course was given to both partners whenever possible The patients were re-examined about ten days after the end of the therapy and all received instructions to return later if signs or symptoms of recurrence appeared.

For statistical analysis χ test was used.

Results

The results of treatment are shown in Table III. The differences from the results achieved in 1961 are statistically highly significant ($p < 0\ 0005$—after one course of treatment χ was 31 75 after two χ was 16 78)

The duration of symptoms did not influence the results of treatment neither did parity age or the menopause In patients with a clinical diagnosis of cervicitis the cure rate was the same as in those cases with a normal cervix (73 per cent-35/48) In ten patients previous treatment with metronidazole had been successful eight of them were cured in this series. Of 13 cases first treated unsuccessfully with nifuratel (*Gjønnæss* and *Aure* 1969) 9 were cured with metronidazole

When the sexual partners could be treated, the cure rate after 2 courses of treatment was 74 per cent (55/74) otherwise it was 75 per cent (33/44) The cure rate in pregnant women was poor 56 per cent (9/16) but the group is too small to demonstrate a statistically significant difference from non pregnant women.

Discussion

A significant decrease in the cure rate of metronidazole treat ment of tricomonal vaginitis from 1961 to 1967 is demonstrated.

The verification of the diagnosis of vaginal trichomoniasis has been discussed. Cytological smears, cultures and wet smear preparations all have their advocates Some investigators have found wet smears to be superior to the other methods (*Bernstine* and *Rakoff* 1953 *Barnes et al* 1957 *Bedoya et al* 1958) Both *Lundström* (1960) and *Svendsen* (1962) found complete

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sibility *Jennison et al* (1961) tested 66 strains of trichomonas for sensitivity to metronidazole seven of them from women who did not respond to treatment. No increased resistance could be demonstrated. Attempts to induce resistance *in vitro* by repeated subcultures have failed (*Ødegaard* 1962 *Watt* and *Jennison* 1962 *Nicol et al* 1966)

Recently *de Carneri* (1966) presented the first report of *in vitro* development of partially resistant strains of trichomonas. After 3 months culture there was a significant increase of tolerance to metronidazole in one strain. With these strains a threefold increase in drug dosage was needed to effect a cure in subcutaneously infected mice In 1966 *Arnold* reported a decrease in cure rates at the Universitatsfrauenklinik in Bern from 92.6 per cent in 1960–1961 to 76 4 per cent in 1965–1966 The author suggested that a certain resistance to metronidazole treatment had developed.

The decrease in cure rates in the present investigation is al most identical with the decrease reported by *Arnold* and as no other reason for this can be seen, the authors have come to the same conclusion.

When the cure rates of treatment of trichomonal vaginitis over the period 1960–1967 have decreased significantly from 97 per cent to 73 per cent, this must be due to development of strains of trichomonas vaginalis totally or partially resistant to metronidazole

SUMMARY

The results of metronidazole treatment of trichomonal vaginitis in 120 cases (200 mg three times a day for 7 days) are reported. Patients in whom this treatment failed were given a second course of treatment. The cure rate after one course of treatment was 61 per cent after two courses 73 per cent. The results of treatment are compared with those obtained in the same hospital in 1961 (97 per cent) A marked decrease in cure rates is noted. The possible reasons for this decrease are discussed. It is sug gested that this decrease must be due to the development of strains of Trichomonas vaginalis resistant to metronidazole.

Table I. Hospital Where and Conditions under Which the Procedures Were Performed

	In-Patients	Out-Patients	Total
County Hospital, Hobro	63	76	139
Central Hospital, Silkeborg	234	0	234
Total	297	76	373

Table II. Nature of Procedure in Relation t Patient Age

	Age				
Nature of Procedure	20	20–39	40–59	≥ 60	Total
Curettage for diagnostic purposes	9	105	185	22	321
Curettage for abortion	9	39	4	0	52
Total	18	144	189	22	373

vaginal plexus (Frankenhauser) which is a cluster of ganglia situated in the parametrium around the main trunk of the uterine artery just lateral to and behind the cervix on a level with the internal os. This blocks the afferent impulses from the uterus to the inferior and superior hypogastric plexuses.

A special 15 cm needle is used mounted on an ordinary 20 cc syringe. A guide may be used, but is not necessary.

The needle is inserted into the vaginal fornices. Many authors have reported two injection sites, at 3 and 9 o clock or at 4 and 8 o clock. Four or more sites of injection have also been used. In the present series 4 injections were made, at 3 6 9 and 12 o clock.

As for the depth of injection, the various reports in the literature quote between 1/2 and 2 cm. In the present series the depth was about 1 cm.

Lidocaine (Leostesin[1] Xylocaine[2]) as well as mepivacaine

Jørgensen, P I Acta obst. et gynec. scandinav. 48, 446, 1969

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PARACERVICAL BLOCK

Use in Curettage for Diagnostic Purposes and for Abortion

BY

P I JØRGENSEN

The deposition of a local anaesthetic around the nerves and nervous plexuses of the paracervical tissue for alleviating or eliminating uterine pain in gynaecological procedures by the vaginal route was described as early as 1909 by *Wertitz*

During the subsequent years the German literature brought several reports of the use of this form of anaesthesia for example for curettage vaginal hysterotomy and hysterectomy (*Thaler* 1912 *Ruge* 1912)

In Denmark *Hansen* in 1948 found paracervical block (p.b.) to be applicable in curettage for legally approved abortions.

In recent years a few American authors have reported good results when using p b for curettage for diagnostic purposes as well as for abortion (*Mengert* and *Slate* 1960 *Connar* and *Bepko* 1964)

But the main use of paracervical anaesthesia is in obstetrics as is apparent from numerous American and now also Scandinavian reports (*Gad* 1967 *Simonsen* 1967)

In the present study an attempt has been made to estimate the efficacy and side effects of p b in 373 patients who had curettage for diagnostic purposes or for abortion using p.b anaesthesia.

Mechanism of Effect and Technique

When inserting a p b the surgeon tries to deposit the local anaesthetic around the paracervical nerves in particular the utero-

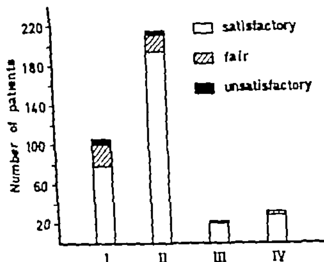


Fig. 1. Analgesic effect in curettage for diagnostic purposes and for abortion with and without premedication. Column *I*: Curettage for diagnostic purposes without premedication. Column *II*: Curettage for diagnostic purposes with premedication. Column *III*: Curettage for abortion without premedication. Column *IV*: Curettage for abortion with premedication.

Efficacy

As premedication about two-thirds of the patients received i.v. injection of pethidine 50 mg, immediately before the insertion of p.b.

The efficacy was estimated after a latent period of 10 minutes and based upon an objective assessment of the patient and her statements concerning freedom from pain during the procedure. By this means, the efficacy could be estimated as

(1) Satisfactory (no or negligible pain)
(2) Fair (moderate, tolerable pain)
(3) Unsatisfactory (severe pain)

Fig. 1 relates the efficacy to the nature of the procedure and the premedication.

In diagnostic curettage without premedication (106 patients) the effect proved satisfactory in 78 (74 per cent), fair in 23

(Carbocaine[R]) may be used. We used Leostesin-noradrenaline[R] 1 % in most cases 20 ml equally distributed in the 4 sites of injection. As a rule it is recommended that a vasoconstrictor should be used as this is supposed to reduce the risk of side effects and to cause less bleeding and more prolonged analgesia.

As a preliminary the vulva and vagina are washed. Thereafter the cervix is examined with a speculum and grasped with a tenaculum so that the fornices are distinctly visible. The injection is made slowly after it has been established by aspiration that the needle has not entered a vessel.

The time from the insertion of the block until the surgical procedure was started was usually about 10 minutes.

Present Material

The series was collected in the Surgical Department of the County Hospital Hobro in February–October 1966, and in the Gynaecological-Obstetrical Department of the Central Hospital, Silkeborg, during the period November 1966 to April 1967 It includes 373 patients, both out patients and in-patients who had curettage for diagnostic purposes or for abortion with p b. as analgesia (Table I)

During the period of the study p b was the preferred method of anaesthesia for the procedures mentioned. Other methods were used only in cases where p b was felt to be less suitable because of anatomical conditions the patients apprehension, suspicion of adnexal inflammation or increased risk of haemorrhage (anticoagulant medication) Apart from these few exceptions (about 10 patients) there was no selection.

Diagnostic curettage was preceded in all cases by dilatation of the cervical canal until it admitted a Hegar 6–7 Some of the patients had a cervical biopsy the removal of a cervical polyp or curettage of the cervical canal at the same time. In curettage for abortion only a quarter of the patients required dilatation of the cervical canal.

Table II gives the nature of the procedures in relation to the patients age.

reduce the frequency of this complication which was relatively most common in the age range 40–59 years (17 per cent)

Precordial pain or palpitations occurred in 5 patients, accompanied in 4 by an elevation of blood pressure In all cases the symptoms soon passed. Two of these patients sufferred from chronic bronchial asthma and hypertension.

The 234 patients admitted to the Central Hospital, Silkeborg, had a routine check of their blood pressure immediately before and after insertion of the block and after the procedure was completed.

An elevation of blood pressure (≥ 20 systolic and/or diastolic) was observed in 74 patients (32 per cent) most commonly in the age range 40–59 years. After the procedure the blood pressure had in most cases returned to initial level.

Among 28 patients with headache 22 (79 per cent) showed elevation of blood pressure as compared with 52 (25 per cent) of the remaining 206 patients who did not have headache.

Nausea occurred in 16 patients, only among those who had received premedication.

Among the somewhat later complications, parametrial bleeding, infection, as well as injuries to nerves or organs might have been expected. In the present series only fever and pelvic pain occurred. These complications were assessed only in the in-patients, as out-patient follow-up was not sufficient for this purpose.

Among patients who had a diagnostic curettage 10 (4 per cent) were febrile for a day or two after the procedure and 5 also had pelvic pain.

After curettage for abortion 11 (21 per cent) had a mild elevation of temperature for the first few days. This group includes 8 patients admitted for incomplete abortion, 6 of whom had fever on admission. The last 3 patients were having legally induced abortion, and none of them suffered pain after the procedure

In all 21 cases the fever had subsided in a few days. Gynaecological examination on discharge did not disclose any changes to explain the febrile reaction. In this connection there is reason to mention a 45-year-old patient who had curettage without any immediate complications and who was discharged 4 days later after gynaecological examination had shown no abnormality She

(22 per cent) and unsatisfactory in 5 (4 per cent) 214 patients had received premedication for the same procedure. The effect was satisfactory in 194 (91 per cent) fair in 16 (7 per cent) and unsatisfactory in 4 (2 per cent) One patient was excluded, because the insertion of p b had to be interrupted because of side effects (see Side Effects)

Without premedication the effect in curettage for abortion was found to be satisfactory in 20 patients unsatisfactory in one. With premedication it was satisfactory in 29 and fair in 2. In no case did dilatation of the cervical canal prior to curettage for abortion elicit pain

Side Effects

Simultaneously with the insertion of p b some patients exhibited side effects in the form of shock like states headache precordial pain or palpitations hypertension or nausea.

After the injection of 18 ml of the local anaesthetic a 73-year old patient turned pale complaining of precordial pain as well as malaise At the same time her blood pressure fell from 170/100 to 70/40 The pulse became soft, but remained regular around 60. No changes in consciousness occurred. After she had been placed in the Trendelenburg position and had received oxygen and dextran the condition soon improved. An emergency ECG as well as an ECG on the following day failed to show any signs of acute changes The procedure had to be given up and the patient is not included in the assessment of the efficacy of this method.

A quarter of an hour after the insertion of the block a 52 year-old patient who was having a diagnostic curettage complained of malaise Her blood pressure fell from 150/90 to 90/40. The pulse was unchanged, full around 60 The malaise soon disappeared after she had been placed in the Trendelenburg position and given oxygen and the procedure was carried through.

Forty-four patients (12 per cent) complained of headache during or immediately after the application of the block In most cases the headache affected the back of the head and usually disappeared in a few minutes The use of premedication did not

signs of incipient inflammation. There was no case of parametrial haematoma as described, one case each, by *Dennis*, (1964) and *Page* (1961) after p.b used during delivery

P.b. requires a minimum of instruments and is relatively easy to insert. An anaesthetist is not needed. In departments having no full-time anaesthetist, in the consulting room, and for patients who do not want general anaesthesia, the method must be considered valuable. In the treatment of patients admitted as emergency cases with profuse bleeding, p.b. has the additional advantage that the patients need not necessarily be fasting and that immediately after the insertion of the block the haemorrhage often decreases appreciably presumably an effect of the noradrenaline.

Its drawbacks are the side effects mentioned above and the inability to check the findings by bimanual examination on a fully relaxed patient.

The only absolute contra indication is allergy to local anaesthetics. Owing to a possibly increased risk of side effects, some caution should be displayed in using the method in patients with hypertension, serious cardiac disease and parametritis, as well as in patients with an increased risk of bleeding.

To meet possible severe side effects, injectable barbiturates as well as apparatus and drugs for resuscitation should always be at hand.

SUMMARY

Paracervical block was used for 373 patients having curettage for diagnostic purposes and for abortion.

With pethidine as premedication, a satisfactory analgesic effect was observed in 90 per cent of the patients.

A 73-year-old patient developed signs of shock during the insertion of the block. 13 per cent exhibited side effects in the form of headache or mild cardio-vascular complaints, usually accompanied by elevation of blood pressure. There were no late complications that could be definitely ascribed to the block.

Summing up it may be said that the method as used in the

was re-admitted 10 days later with fever and pelvic pain. Laparotomy revealed a large right tubo-ovarian abscess.

On the day after the procedure one patient developed a generalized skin rash which responded to 2 days antiallergic therapy (Antistina® tablets)

No patient complained of bladder or bowel disturbances or of neurological symptoms.

Discussion

The object of this study was to assess the applicability of p.b in curettage of the uterus.

The analgesic effect with pethidine given simultaneously as premedication was satisfactory in more than 90 per cent of the patients. Others have obtained similar results *Hansen*, in 1948, found good analgesia in 89 out of 90 patients who had dilatation of the cervical canal and curettage for abortion after insertion of p b *Mengert and Slate* 1960 used p b. in 377 out-patients who had dilation of the cervical canal and curettage for diagnostic purposes. No premedication was given. The analgesic effect was good in about 90 % of the patients. *Connar* and *Bepko* 1964 treated 605 patients by curettage for abortion, using paracervical-pudendal block after barbiturate premedication. The analgesic effect was satisfactory in all cases but one

The incidence of side effects immediately after the injection was relatively high. Thus 44 patients (12 per cent) developed headache and 5 cardiac symptoms. The fact that in most cases these complaints were accompanied by elevation of blood pressure renders it likely that noradrenaline was responsible Apart from one of the two patients who exhibited a shock like state, however there were no serious side effects during the insertion of p b It may be mentioned that in the literature on p b. no similar side effects have been reported except for a single case of "hypotensive reaction for which only a few details are given (*Mengert* and *Slate* 1960)

None of the late complications was serious. Nearly all were

signs of incipient inflammation. There was no case of parametrial haematoma as described, one case each, by *Dennis*, (1964) and *Page* (1961) after p.b used during delivery

P.b. requires a minimum of instruments and is relatively easy to insert. An anaesthetist is not needed. In departments having no full-time anaesthetist, in the consulting room, and for patients who do not want general anaesthesia, the method must be considered valuable. In the treatment of patients admitted as emergency cases with profuse bleeding, p.b has the additional advantage that the patients need not necessarily be fasting and that immediately after the insertion of the block the haemorrhage often decreases appreciably presumably an effect of the noradrenaline.

Its drawbacks are the side effects mentioned above and the inability to check the findings by bimanual examination on a fully relaxed patient.

The only absolute contra-indication is allergy to local anaesthetics. Owing to a possibly increased risk of side effects, some caution should be displayed in using the method in patients with hypertension, serious cardiac disease, and parametritis, as well as in patients with an increased risk of bleeding.

To meet possible severe side effects injectable barbiturates as well as apparatus and drugs for resuscitation should always be at hand.

SUMMARY

Paracervical block was used for 373 patients having curettage for diagnostic purposes and for abortion.

With pethidine as premedication, a satisfactory analgesic effect was observed in 90 per cent of the patients.

A 73-year-old patient developed signs of shock during the insertion of the block. 13 per cent exhibited side effects in the form of headache or mild cardio-vascular complaints, usually accompanied by elevation of blood pressure There were no late complications that could be definitely ascribed to the block.

Summing up it may be said that the method as used in the

present series proved simple cheap and sufficiently effective. However side effects were relatively common but generally not serious.

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MEASUREMENT OF HUMAN UTERINE CERVICAL BLOOD FLOW BY LOCAL HYDROGEN GAS CLEARANCE

BY

INGE KLINGENBERG AND KNUT AUKLAND

Present knowledge of local blood flow in the human myometrium is based on relatively few observations. Total uterine blood flow has been measured by electromagnetic flowmeter (*Assali et al.* 1960) and by N_2O-technique (*Assali et al.* 1953 *Metcalfe et al.* 1955) but no information on local blood flow in the myometrium can be obtained by these methods. A relative measure of local myometrial blood flow may be obtained by heat conductivity (*Prill* 1959) or local clearance of Na^{24} (*McCall* and *Sopp* 1951) but neither method gives an absolute flow measurement. Theoretically local clearance of $Xenon^{133}$ injected into the myometrium can be interpreted in terms of absolute flow per gram of tissue and measurements have been described both for isthmus and cervix in pregnant and non pregnant women (*Munck et al.* 1964 *Lysgaard* and *Lefèvre* 1965)

This article presents data on local blood flow in the fibromuscular tissue of the cervix in humans measured by the hydrogen desaturation technique (*Aukland et al.* 1964). The criteria for acceptable measurements are discussed on the basis of studies on the reproducibility of subsequent measurements in a given area, and on comparison of simultaneous measurements in different areas of the cervix.

time will therefore give a linear desaturation curve with a slope determined by k. The numerical value of k is conveniently determined from the half time, $T_{\frac{1}{2}}$ which is the time required for tissue concentration to be reduced to half its numerical value. Equation (4) may thus be written

$$\ln C_t = \ln C_o - kt$$

or

$$k = \frac{\ln C_o - \ln C_t}{t} \tag{5}$$

For 50 % reduction of concentration

$$k = \frac{\ln 100 - \ln 50}{T_{\frac{1}{2}}} = \frac{0.693}{T_{\frac{1}{2}}}$$

Since $k = \frac{F}{W\lambda}$

$$\frac{F}{W} = \frac{0.693\ \lambda}{T_{\frac{1}{2}}} \tag{6}$$

As the tissue/blood partition coefficient is close to unity in such different tissues as kidney (*Aukland et al.* 1964) and brain (*Fleschi et al.* 1964) the myometrium/blood partition coefficient is also assumed to be 1.00 Therefore

$$\frac{F}{W} = \frac{0.693}{T_{\frac{1}{2}}} \tag{7}$$

If $T_{\frac{1}{2}}$ is measured in minutes local flow F/W is obtained in ml/min per gram tissue

Fig. 1 illustrates schematically the polarographic method for measuring hydrogen concentration When a platinum electrode placed in saline is connected to a silver/silver chloride reference lectrode and polarized by a dry cell at a potential of +0.3 volts relati e to the reference only minimal current is produced in the circuit If hydrogen gas is then added to the solution, hydrogen

Methods

Local myometrial blood flow was measured by polarographic recording of hydrogen gas desaturation (*Aukland et al.*, 1964). The method is based on the theory of *Kety* (1951) for gas exchange between blood and tissue. According to *Fick's* principle, the amount of test substance brought to an organ by arterial blood is equal to the sum of the amount of the test substance metabolized the amount accumulated in the organ and the amount removed by venous blood. The present test substance, hydrogen gas is not metabolized It is assumed that tissue blood volume is unchanged during the investigation period and that blood flow (F) is constant.

If the concentration of hydrogen in arterial blood is C_a, in venous blood C_v and the hydrogen concentration change dC_t in the tissue volume W during the time dt, the Fick principle gives the equation

$$dC_tW = FC_adt - FC_vdt \tag{1}$$

Because of the high diffusibility of hydrogen gas, one may assume diffusion equilibrium between tissue and venous blood during the saturation and desaturation period. If the tissue/blood partition coefficient for hydrogen is $\lambda = C_t/C_v$ equation (1) becomes

$$dC_tW = F(C_a - C_t/\lambda)\,dt \tag{2}$$

When arterial hydrogen concentration is suddenly lowered to zero ($C_a = O$) equation (2) yields

$$dC_t = \frac{C_tF}{W\lambda}\,dt \tag{3}$$

Integration between the limits t = O and t = t yields

$$C_t = C_{t0}e^{-kt} \tag{4}$$

where C_{t0} is the initial tissue concentration and $k = \frac{F}{W\lambda}$ showing that tissue concentration falls as an exponential function of time. Hydrogen concentration plotted on a logarithmic scale against

time will therefore give a linear desaturation curve, with a slope determined by k. The numerical value of k is conveniently determined from the half time, $T_{\frac{1}{2}}$ which is the time required for tissue concentration to be reduced to half its numerical value. Equation (4) may thus be written

$$\ln C_t = \ln C_\infty - kt$$

or

$$k = \frac{\ln C_\infty - \ln C_t}{t} \quad (5)$$

For 50 % reduction of concentration

$$k = \frac{\ln 100 - \ln 50}{T_{\frac{1}{2}}} = \frac{0.693}{T_{\frac{1}{2}}}$$

Since $k = \frac{F}{W\lambda}$

$$\frac{F}{W} = \frac{0.693\ \lambda}{T_{\frac{1}{2}}} \quad (6)$$

As the tissue/blood partition coefficient is close to unity in such different tissues as kidney (*Aukland et al.* 1964) and brain (*Fieschi et al* 1964) the myometrium/blood partition coefficient is also assumed to be 1.00 Therefore

$$\frac{F}{W} = \frac{0.693}{T_{\frac{1}{2}}} \quad (7)$$

If $T_{\frac{1}{2}}$ is measured in minutes local flow F/W is obtained in ml/min per gram tissue

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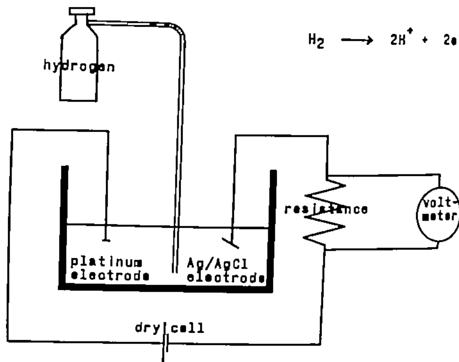


Fig. 1 Principle of hydrogen polarography

molecules will be oxidized at the platinum surface, thereby producing a current proportional to the concentration of dissolved hydrogen gas.

Measurements in the cervical fibromuscular tissue were performed with needle-shaped electrodes made of 0 8 mm thick platinum wire. The 20–30 mm long electrodes were insulated with lacquer except for the 1 5 mm-long pointed tip, which was left bare The silver/silver chloride electrode was formed as a 2.6 × 3 3 cm sheet and fixed by adhesive tape to the skin prepared with electrolyte ointment. The electrode current was measured with a Keithley 150 A microvolt ammeter and recorded on a Honeywell Brown electronic recorder (Fig 2) With a 6-way switch, six electrode circuits could be read alternately without changing the polarizing potential (*Aukland et al.* 1967)

The electrically insulated patient lay in bed during the investigation. No anaesthetics or other drugs were used With a speculum in the vagina, the cervix was grasped with volsella and two or

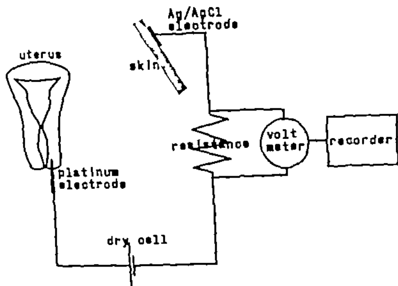


Fig 2 Hydrogen polarography applied to uterine myometrial blood flow measurement

three platinum electrodes were inserted into the cervix parallel to the cervical canal. The depth of electrode insertion was measured in each case, but the exact position relative to the cervical canal was difficult to assess. By probing the cervical canal with a sound, the electrode tip was sometimes found to have penetrated to the mucosa or submucosa of the canal. Measurement from such electrode positions were discarded. After electrode insertion, the volsella and speculum were removed.

Hydrogen gas was added to the respiratory air by open mask, giving an alveolar hydrogen concentration of 5–15 volume per cent. When the uterine cervical tissue had reached a steady concentration, indicated by the electrode current levelling off the administration of hydrogen was stopped and the patient asked to hyperventilate forcefully for approximately 20 seconds. Hydrogen was thereby rapidly removed from the alveoli and arterial blood, and cleared from the cervical tissue at a rate proportional to local blood flow The current produced by the platinum electrode

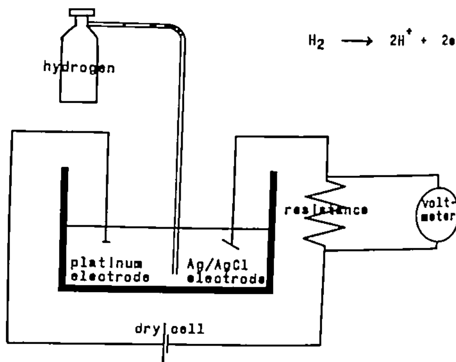


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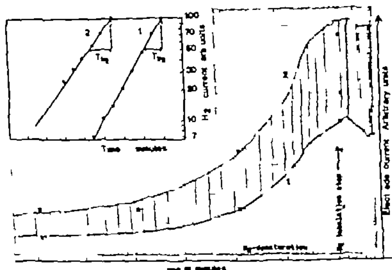


Fig. 3 Hydrogen desaturation curves recorded simultaneously from two different electrodes in the cervix of 52-year-old woman with irregular uterine bleeding. Inset semilogarithmic plot of the desaturation curves. Curve 1 monoexponential, curve 2, multiexponential. Straight lines show the slopes used for flow calculation.

3. Each tissue compartment is represented in the summation curve according to its weight (*Ingvar* and *Lassen* 1962).

The second assumption was satisfied by sufficiently long equilibration time but the first and third assumptions may not be valid, as discussed by *Aukland* et al. (1967) and may cause too high a flow estimate.

To some extent this conclusion can be evaluated empirically from the present data. Monoexponential curves were obtained in 109 measurements in 45 patients, and multiexponential curves in 107 measurements in 44 patients (in some patients, both mono- and multiexponential curves were obtained). The mean age in the monoexponential group was 43.3 and in the multiexponential 44.1. Table 1 shows that the two groups were also comparable with respect to diagnosis. Fig. 4 indicates that flow distribution was similar in the two groups although flow values from mono-

gave a continuous indication of the hydrogen concentration in the tissue.

The first measurement was undertaken 20–30 minutes after insertion of the electrodes. The second measurement was performed 20–30 minutes later and in some cases blood flow was measured up to 4½ hours after electrode insertion.

Results and Discussion

A total of 216 desaturation curves were recorded from 116 different electrode sites in 60 patients aged 20 to 71 years the majority being between 30 and 55 years. A further thirteen curves were excluded for technical reasons.

Original desaturation curves recorded simultaneously from two electrodes placed in the anterior lip of the cervix of a 52 year old woman suffering from irregular bleeding, are reproduced in Fig 3 lower right part. A semi logarithmic plot of the same curves is given in the upper left part of the same figure. One of the electrodes showed a good monoexponential desaturation as predicted theoretically for homogenous blood flow in the small tissue volume surrounding the electrode tip (Cf equation 4) The other curve shown in Fig 3 clearly deviates from monoexponential desaturation and this was a relatively frequent finding. To evaluate the significance of the different curve shapes, all curves were divided arbitrarily into monoexponential and multiexponential the criterion for monoexponential being a practically linear semi logarithmic plot down to at least 80 per cent desaturation It is obvious that this criterion leaves considerable margin for subjective judgment but we were unable to define more strict criteria for routine use.

For multiexponential curves blood flow was calculated from the initial slope of the desaturation curve This procedure is theoretically correct, provided that

1 The multiexponential desaturation is due to nonhomogeneous capillary blood flow and accordingly different desaturation rates in the tissue surrounding the electrode
2. All tissue compartments are in parallel and completely equilibrated with arterial blood at the start of desaturation, and

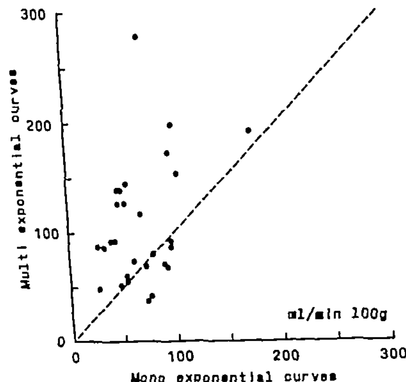
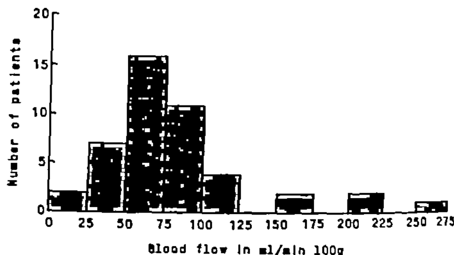


Fig 5 Comparison between simultaneous measurements with two electrodes giving mono- and multiexponential curves respectively

multiexponential curves gives too high an assessment of absolute flow per gram of tissue

Simultaneous measurements at different electrode sites showed considerable scatter among monoexponential curves, as is evident from Fig 6 Multiexponential curves showed even greater differences The standard deviation for monoexponential curves, judged from simultaneous measurements, was 20.3 per cent of their mean, for simultaneous multiexponentials 30 4 per cent, and for mono-multiexponential pairs 42.3 per cent. Even with mono-



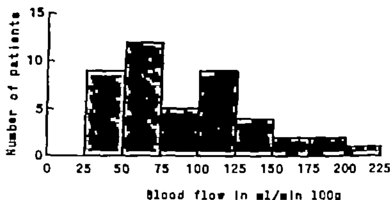


Fig. 4 Distribution of blood flow in 60 patients. Mean flow for each patient obtained from 1–3 measurements, each with 1–3 electrodes. Upper part: Monoexponential curves (45 patients) Lower part Multiexponential curves (44 patients)

exponential curves showed a somewhat closer grouping around the mean.

As is evident from Fig 5 where simultaneous measurements from different electrode sites are compared multiexponential curves gave on average higher flow rates than monoexponential 109 and 75 ml/min 100 g respectively As the monoexponential curves do not present theoretical problems in calculating blood flow it seems reasonable to conclude that the initial slope of

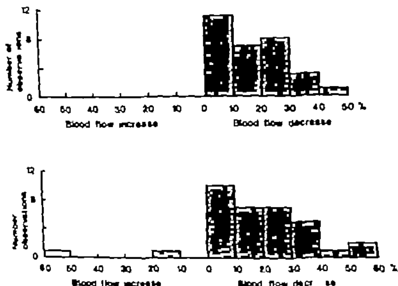


Fig. 7 Percentage change in blood flow from first to second measurement at the same electrode site. Upper part: Monoexponential curves. Lower part: Multiexponential curves.

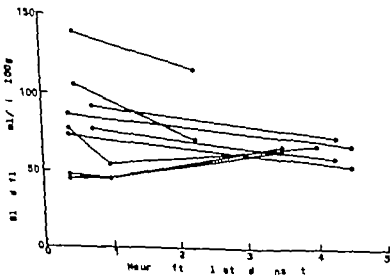


Fig. 8 Blood flow measurements at different times after electrode insertion

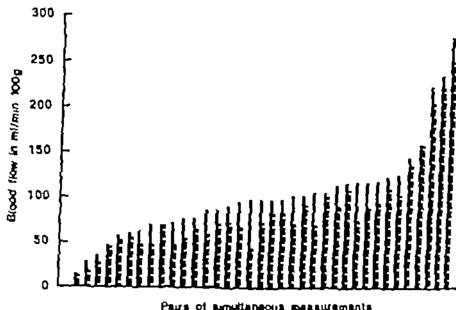


Fig. 6. Comparison between pairs of monoexponential curves obtained in simultaneous measurements.

exponential curves the scatter is about twice as great as for measurements in the myocardium of dogs, using the same technique (*Aukland et al.*, 1967) This suggests that to large extent the variations reflect real flow differences between various areas of the cervix. No consistent flow differences were observed between anterior or posterior lip, nor between varying depths of electrode implantation in the range of 5–20 mm. It seems likely however that the mixture of muscle and connective tissue in the human cervix may be the cause of more heterogenous blood flow than was observed in the myocardium of dogs, and that this is reflected both in the frequent occurrence of multiexponential desaturation curves and the considerable scatter between results from different electrode positions. Scar tissue and other pathological changes might also add to variability

The conclusion that the scatter between different electrode sites is mainly due to local differences and not to methodological errors, is also supported by the good reproducibility with repeated measurements from electrodes in the same place. However flow estimated by the first measurement, was generally higher than

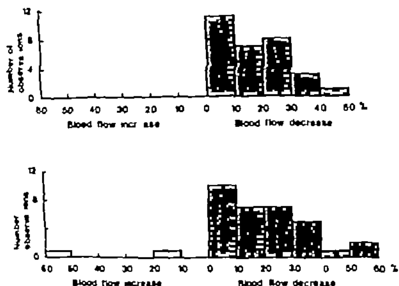


Fig 7 Percentage change in blood flow from first to second measurement at the same electrode site Upper part Monoexponential curves Lower part Multiexponential curves

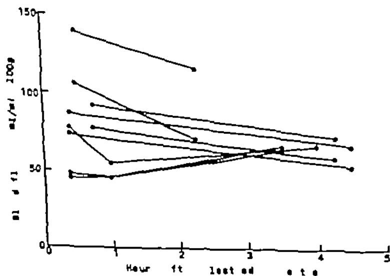


Fig 8 Blood flow measurements at different times after lectrode insertion

that in the second measurement 20–30 minutes later As is evident from Fig. 7 which shows percentage changes from first to second measurements this tendency was clear both from mono- and multiexponential curves although the scatter was greater in the latter This observation suggests that multiexponential curves may also show relative changes in local blood flow but should probably not be interpreted in terms of absolute flow Repeated measurements up to about 4 hours after electrode insertion (Fig. 8) showed a mean decrease of 9 per cent from the first measurement, suggesting that no consistent flow changes take place after the first hour of electrode insertion. It seems probable that the manipulation of the cervix during insertion of the electrodes might induce transitory changes in blood flow and that the second measurement is more representative of physiological blood flow

Monoexponential curves were obtained in 45 of 60 patients, and the following discussion on absolute blood flow includes only these measurements (Table I) The mean flow rate for individual patients calculated from one to three measurements each with 1–3 electrodes gave flow values ranging from 13 to 264 ml/min 100 g. Most measurements were between 50 and 100 ml/min 100 g, and had a mean of 83 ml/min 100 g. Five patients had cervical blood flow greater than 150 ml/min 100 g Three of these had uterine myomata and their blood flow was 153 212 and 221

Table I *Diagnosis and Types of Desaturation Curves*

Diagnosis	Number of Patients		
	Total	Monoexp. Curves	Multiexp. Curves
Pregnancy 6–12 weeks of gestation	8	8	6
Metrorrhagia	20	13	17
Uterine myomata	12	10	8
Carcinoma of cervix or corpus	5	4	1
Uterine prolapse	4	2	2
Vaginal prolapse	4	3	4
Other	7	5	6
	60	45	44

Table II. *Blood Flow Obtained from Monoexponential Curves*

Diagnosis	Number of Patients	Mean Age (Years)	Mean Blood Flow ± S.E. ml/min 100 g
Pregnancy 6–12 weeks of gestation	8	33.7	69.4 ± 6
Metrorrhagia	13	41.1	73.4 ± 5.8
Uterine myomata	10	47.4	116.6 ± 19.2
Carcinoma of cervix or corpus	4	49.0	66.8 ± 9.0
Uterine prolapse	2	57.0	25.5
Vaginal prolapse	3	53.7	78.0
Other	5	40.2	117.6
Total	45	43.3	82.9 ± 7.9

ml/min 100 g respectively. A 20-year-old girl with a normal genital tract had a mean blood flow value of 264 ml/min 100 g, and in one patient admitted for uterine bleeding where a curettage had been performed the day before measurement, blood flow was found to be 173 ml/min 100 g. The lowest blood flow (13 ml/min 100 g) was obtained in a patient aged 71 who had a vault prolapse following a subtotal hysterectomy. Cervical blood flow in early pregnancy was found to be about the same as in non-pregnant women. Two older patients with uterine prolapse both had low cervical blood flow (23 and 28 ml/min 100 g). Patients with uterine myomata had significantly higher cervical blood flow than the rest of the patients ($p = 0.05$) (Table II). Otherwise as shown in Table II, the wide variations were not clearly correlated to diagnoses. Furthermore no correlation was observed between cervical blood flow and age or parity.

Considerably lower values have been obtained by external recording of clearance of Xenon[133] injected into the myometrium. In three non-pregnant women *Munck et al.* (1964) found cervical blood flow ranged from 9 to 14 ml/min 100 g, and isthmic flow between 15 and 35 ml/min 100 g. They observed values between 4 and 37 ml/mm 100 g in the body of pregnant uteri. *Lysgaard and Lefèvre* (1965) using the same technique reported blood flow varying between 2 and 32 ml/min 100 g in the myometrium during pregnancy. *McCall* and *Sopp* (1951) found clearance

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constants for Na^{24} of 0 04 to 0 65 min $^{-1}$ but since Na^{24} is not freely diffusible across cell membranes sodium clearance cannot be interpreted in terms of absolute blood flow

The cervical blood flow obtained by the present technique was indeed higher than expected. It is difficult to understand why the cervical tissue should have a blood flow as high as myocardium (Gregg, 1963) or brain (Kety 1960) We believe however that errors which could result in falsely high values such as too short saturation time and multiexponential desaturation curves, have been excluded The tissue/blood partition coefficient remains to be determined for the myometrium, but it seems unlikely that this could alter the calculated flow significantly

The main advantages of the hydrogen desaturation method are that blood flow can be measured simultaneously in different places and that measurements can be repeated with high reproducibility in a given area. The measurements are easily carried out, are without complications and involve only slight discomfort to the patient

SUMMARY

Blood flow was measured in the human uterine cervix by local clearance of hydrogen gas recorded from needle-shaped platinum electrodes inserted into the cervical tissue. A description is given of the theoretical basis for the method and the practical performance of the measurements

Repeated measurements from a given electrode site showed good reproducibility whereas simultaneous measurements from different electrode sites showed considerable scatter (SD 20 per cent) probably reflecting local differences in blood flow Acceptable absolute flow measurements were obtained in 45 out of a heterogeneous series of 60 patients and gave a mean flow of 83 ml/min 100 g The average cervical blood flow in eight women in early pregnancy was 69 ml/min 100 g This was not significantly different from that in the remaining cases in the series. The highest average flow was found in patients with uterine myomata

The main advantages of the method are that local blood flow can be measured a) repeatedly at the same site, and b) at different sites simultaneously

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MEASUREMENT OF BLOOD FLOW IN HUMAN MYOMETRIUM BY LOCAL HYDROGEN CLEARANCE

BY

INGE KLINGENBERG

Measurement of blood flow in the human uterine cervix by a hydrogen clearance technique has been reported previously (*Klingenberg* and *Aukland* 1969) In order to perform similar measurements in the human myometrium a special instrument was constructed, permitting simultaneous measurements from two different areas of the myometrium. The instrument was tested in 27 unanaesthetized nonpregnant women and gave technically satisfactory measurements in all except 5 cases. The observed myometrial blood flow was compared to simultaneous measurements in cervical tissue

Methods

A 3 cm long, needle-shaped, lacquer insulated platinum wire with a diameter 0 8 mm is soldered to a teflon-insulated copper wire and fixed in place in a steel tube with epoxyresin (Araldite Ciba) (Fig. 1) The steel tube has an outer diameter of 1.5 mm and a length of 21 cm. The lacquer insulation on the 2.5 mm long free end of the tapered platinum wire is removed. Two such electrode tubes are soldered together side by side up to a point 4.5 cm from the electrode tips. In the resting position the electrode tips are 1.5 cm apart, but can be pressed together and kept in this position by a slide. The other end of the united electrode tubes is soldered to a 2.5 cm long steel cylinder provided with screw threads for a

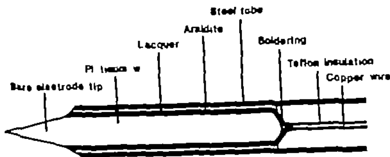


Fig. 1 Mounting of platinum electrode in steel tube.

stop plate, which can be adjusted by means of two nuts. The electrode tubes fit into an introducer made of a steel tube with outer diameter 4 mm. One end of the introducer is closed and rounded, and 3 cm from this blunt end there are two slits for the electrodes (Fig. 2, upper part) The electrodes are inserted into the introducer with the electrodetips pressed together by the slide. During insertion, the slide is retracted by the open end of the introducer allowing the electrodetips to emerge through the slits in the introducer (Fig. 2, lower part) By means of the stop plate and the nuts, the position of the electrode tips can be regulated relative to the blunt end of the introducer A globe chain, which fits into a track in the stop plate is soldered to a small tenaculum forceps.

During insertion of the instrument the patient is lying in lithotomy position, electrically insulated. No anaesthetics or other drugs are used. The cervix is exposed with a speculum cleansed and pulled down with the small tenaculum forceps. With the electrodes shielded in the introducer the instrument is introduced through the cervical canal until it touches the fundus of the uterus The electrode tubes are then pushed forwards so that the myometrium is impaled by the electrodes. The electrodes are kept in this position by the small tenaculum forceps, which is locked tight to the stop plate by the globe chain. In most measurements, the electrode tips were adjusted to project 3–4 mm beyond the end of the introducer For simultaneous measurements in the myometrium of the fundus and the cervix, the electrodes for

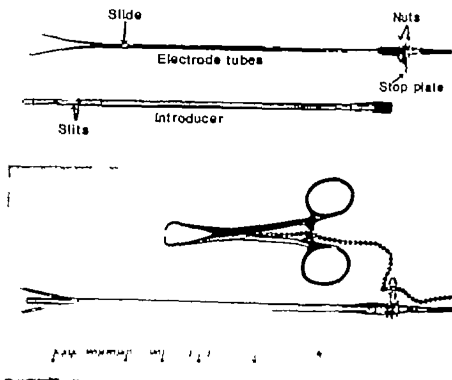
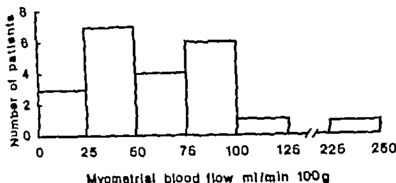


Fig. 2. Instruments for myometrial blood flow measurement.

cervical recordings were inserted before introducing the myometrial instrument. Measurements of hydrogen gas clearance were not started until 20–30 minutes after insertion of the electrodes. The polarographic determination of hydrogen has been described previously (*Klingenberg* and *Aukland* 1969) and the same technique was used in this investigation.

Material

Blood flow measurements in the myometrium were performed in 27 women and simultaneous cervical measurements were obtained in 21 women. The age range was from 20 to 78 years, and the diagnoses are shown in Table I.



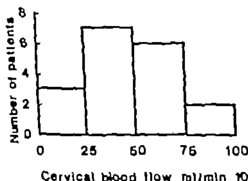


Fig. 3. Upper part: Distribution of myometrial blood flow in 22 patient. Lower part: Distribution of cervical blood flow in 18 patients.

Results

A total of 56 hydrogen desaturation curves was recorded from the myometrium in 27 patients. Thirty-nine curves in 22 patients wer monoexponential to at least 80 per cent desaturation. In 17 patients, monoexponential curves were obtained from both electrodes in the myometrium, and accordingly one of the two desaturation curves was monoexponential in 5 patients. In 2 patients, recordings from one of the electrodes failed for technical reasons, and multiexponential curves were obtained from the other electrode. Both myometrial desaturation curves were multiexponential in 3 other patients in one of them also after epeated measure-

Table I *Average Myometrial and Cervical Blood Flow*

Diagnosis	Number of Patients	Mean Age	Mean Blood Flow ml/min 100 g	
			Myometrium	Cervix
Uterine and vaginal prolapse	5	63	53	32
Metrorrhagia	4	54.5	57	49
Uterine myomata	3	48.5	98	53
Other	5	36.4	50	57

ments had been made with the electrodes in the same position. Since multiexponential curves are difficult to interpret (*Klingenberg* and *Aukland* 1969) the results presented in this paper are based solely on monoexponential curves

The distribution of mean blood flow obtained from 1-2 electrodes in the myometrium of 22 patients is shown in Fig. 3, upper part giving an average of 64 2 ml/min 100 g In one patient, blood flow was found to be 225 ml/min 100 g, the others had blood flow varying from 22 to 109 ml/min 100 g. As is evident from Table I no consistent flow differences were observed between groups of patients with different diagnosis. The high mean flow in the group with uterine myomas is due to high readings (225 ml/min. 100 g) in one patient.

Simultaneous measurements in the myometrium showed good agreement, as illustrated in Fig. 4 where values from two electrodes are placed at random on the abscissa and ordinate respectively

Cervical measurements in 18 patients (Fig. 3 lower part) showed a flow distribution similar to that in the myometrium. Average flow was 47 2 ml/min 100 g, with a range of 17 to 98 ml/min 100 g In patients with uterine prolapse cervical flow was found to be relatively low Otherwise no correlation between cervical flow and age or diagnosis could be demonstrated.

Simultaneous measurements in the myometrium and cervix were performed in 17 patients. As is evident from Fig. 5 the relationship between myometrial and cervical blood flow varied

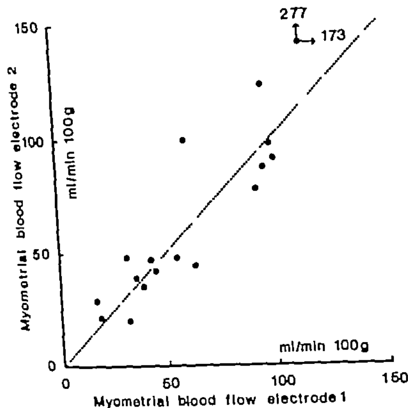


Fig. 4 Comparison between two simultaneous blood flow measurements in the myometrium.

considerably between patients. However if the patients with uterine prolapse are excluded, average myometrial and cervical flow were about the same

Discussion

The instrument described for insertion and fixation of electrodes in the myometrium was found to work satisfactorily gi ing technically good hydrogen desaturation curves from the tissue The depth of electrode insertion into the myometrium may be regulated, but cannot be exactly localized. Most electrodes used

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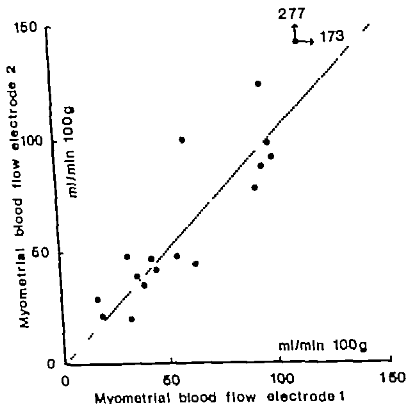


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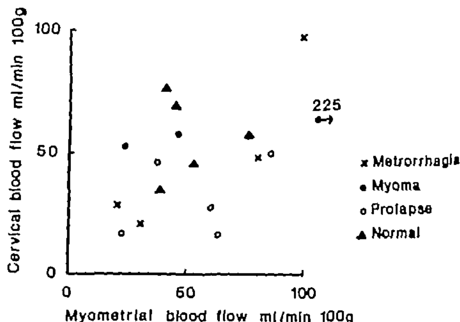


Fig 5. Relationship between blood flow in the myometrium and cervix. Diagnosis indicated by various symbols.

in this investigation were inserted at least 4 mm into the wall, and should therefore have penetrated into the myometrium. However it is possible that due to some irregularity in the shape of the uterine cavity an electrode may have been positioned in the endometrium. The instrument can be introduced through a cervical canal of 4 mm diameter In parous women, the instrument may be inserted without dilatation of the cervical canal. The measurements were performed with only slight discomfort to the patients and no complications were observed.

Whereas the frequency of monoexponential curves from cervical measurements was about 50 per cent (*Klingenberg and Aukland* 1969) 70 per cent of the recordings from the myometrium were monoexponential This may be due to better electrode fixation in the myometrium than in the cervix, and the higher probability of placing the electrodes in the myometrium. The observation may also indicate that blood flow is more homogeneous in the myometrium than in the cervical tissue

Simultaneous blood flow recordings from two areas in the

myometrium usually showed good agreement. Compared with previous investigations of cervical blood flow (*Klingenberg* and *Aukland* 1969) the myometrial scatter was considerably smaller. This suggests that the myometrium has more homogeneous blood flow than the cervix, which is often scarred. The myometrium is probably not completely homogeneous, and some flow variations are therefore to be expected. In the patients in whom relatively large flow differences were observed, one of the electrodes might have been positioned in the endometrium or have been inserted into the peritoneal surface. In contrast, the small difference between simultaneous recordings obtained in most of the patients indicates that both electrodes had been inserted into homogeneous tissue. Since only monoexponential curves were used, it is unlikely that large variations in flow should reflect methodological errors.

As is evident from Table I the blood flow in the myometrium and cervical tissue was of the same magnitude. For unknown reasons the average cervical blood flow obtained in the present patients was somewhat lower than that observed in a previous series (*Klingenberg* and *Aukland*, 1969).

Myometrial blood flow showed great variations between patients. The variations in flow obtained in the present study may to some extent be due to differences in diagnosis or age of the patients. However, no correlation between flow and diagnosis or age was demonstrated in this material. Variations in flow during the menstrual cycle have not yet been studied. A similar scatter has also been obtained by local Xe^{133}-clearance technique. In 5 non-pregnant women, *Munck et al.* (1964) found that isthmic flow ranged from 15 to 35 ml/min 100 g, and flow in the myometrium of pregnant women varied from 4 to 32 ml/min 100 g. *Lysgaard* and *Lefèvre* (1965) reported flow values from 2 to 32 ml/min 100 g in women in the last trimester of pregnancy using the same method. With a new instrument for application of Xe^{133} *Lysgaard* (1966) reported values varying between 4 and 22 ml/min 100 g.

Both the present and a previous study (*Klingenberg* and *Aukland* 1969) suggest that hydrogen clearance gives higher values than Xe^{133}-clearance in both the myometrium and the cervix.

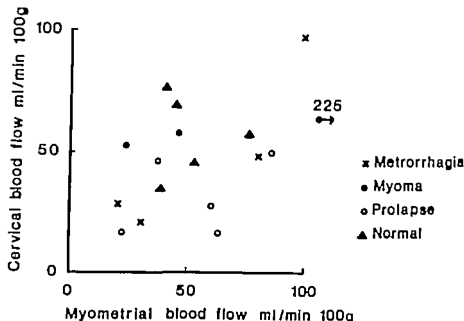


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Lindgren, L. and Holmlund, D. Acta obst. et gynec. scandinav. 48, 478, 1969

From the Department of Obstetrics and Gynaecology (Prof. Per Lundström) and the Department of Surgery (Prof. Stig Borgström), University of Umeå, Sweden

FRICTION BETWEEN THE FOETAL HEAD AND THE CERVIX DURING LABOUR

BY

L. LINDGREN AND D. HOLMLUND

The factors affecting the movements of the foetal head in vertex presentation during labour have been studied by *Rydberg* (1954). His conclusion was that the shape of the foetal head is a major factor in determining its moving during labour. Internal rotation takes place at different stages during the passage through the pelvis but usually begins when the head approaches the pelvic floor after it has become well flexed. If there is a rotation or not during contraction which causes a gliding between the foetal head and the uterine wall has not been studied as we know from the literature. From *Siener's* (1959, 1963) investigations we know that during contraction the foetal head is pressed against the lower uterine segment at the same time as the cervix dilates. From these observations the conclusion can be drawn that there is a gliding movement between the foetal head and the uterine wall during contractions. *Siener* has also demonstrated that during contraction there is a longitudinal tension in the uterine wall. It is evident that during the contraction there is a friction between the foetal head and the cervical wall, which opposes the moving of the foetal head. It is also evident that the friction must be of great importance for the mechanism of the cervical dilatation during spontaneous delivery and during vacuum extraction when the cervix is not fully dilated.

According to the law of Coulomb the friction (F) is the pro-

Although the difference might partly be due to different clinical material, it seems likely that the discrepancy is mainly of methodological origin. As discussed in the previous study acceptance of only monoexponential hydrogen desaturation curves is believed to exclude falsely high values. Furthermore preliminary measurements of blood flow in the uterine artery with an electromagnetic flowmeter have given results compatible with the flow obtained by hydrogen clearance.

SUMMARY

Blood flow in the human myometrium has been measured by local clearance of hydrogen gas using an instrument that can be introduced through the cervical canal without anaesthesia. The measurements were performed with only slight discomfort to the patients Monoexponential hydrogen desaturation curves that can be used directly in estimation of myometrial flow were obtained in 22 of 27 women with various gynaecological conditions. Simultaneous measurements from two electrodes introduced into the myometrium showed good agreement but flow values varied widely between patients with an average of 64.2 ml/min 100 g. No significant differences in myometrial blood flow were observed between patients with uterine prolapse, metrorrhagia and uterine myomata.

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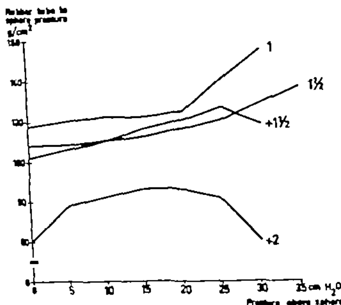


Fig 2 Graph of the sphere to rubber tube pressure at different planes of the sphere when increasing pressure was applied above the sphere
+1 1 2=1 1/2 cm above the equator   1=1 cm below the equator
The curv (+2) is achieved when the tokograph was applied 2 cm above the equator but with its sensitive part, the cylinder towards the sphere

In order to understand the importance of the pressure conditions and the area of contact between the foetal head and the cervix some model studies have been done. A wooden sphere (8 cm in diameter) corresponding to the foetal head was placed in a rubber tube (6.5 cm in diameter) corresponding to the cervical canal. The pressures between the sphere and the rubber tube at different levels planes on the surface of the sphere were measured by the method for intrauterine tocometry (*Ingelman-Sundberg* and *Lindgren* 1955) with different traction forces obtained by means of weights (Fig. 1) At all planes the pressure increased with increased traction but the greatest increase was above the equator of the sphere The friction is thus increased both as a result of the increased pressure between sphere and rubber tube above the equator and because of an increased area of contact.

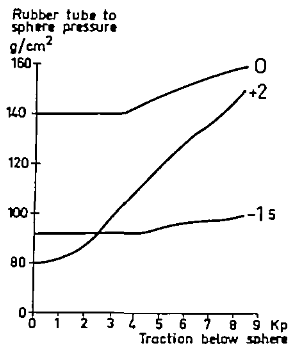


Fig. 1 Graph of the sphere to rubber tube pressure at different planes of the sphere when increasing traction was applied below the sphere.
0 = Equator +2 = 2 cm above the equator -1.5 = 1.5 cm below the equator

duct of the coefficient of friction (f) and the force which presses two objects together (N)

(1) $F = f \times N$

During contraction the friction between foetal head and cervical wall is increased by the driving force which forces the foetal head downwards. This driving force (F) is the product of the amniotic fluid pressure (P) and in most cases, the cross-section at the largest circumference of the foetal head.

(2) $F = P \times \pi R^2$

The factor N in equation (1) is the product of the head to cervix pressure (Q) and the head to cervix area (a)

(3) $N = Q \times a$

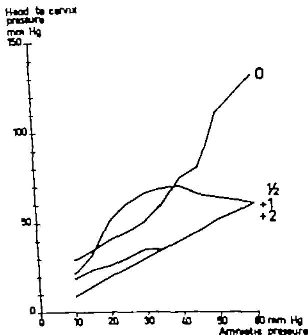


Fig 4 Graph of the head to cervix pressure during contraction. 0 = largest circumference of the foetal head +1/2, +1 +2 stands for 1/2, 1 and 2 cm above this level Two cm above the largest circumference of the foetal head ther was no contact between t and the cervical wall.

largest circumference of the foetal head and decreases at the presenting part of the head. During uterine contractions the head to cervix pressure is higher than the corresponding amniotic fluid pressure This fact explains the engagement of the foetal head in the uterus. During contractions the head to cervix pressure above the equator of the head increases very rapidly (Fig 3) and is sometimes greater than the increase in pressure at the equator (Fig. 4) With further increase in the amniotic fluid pressure the head to cervix pressure at the equator decreases. The amniotic fluid pressure is measured at maximum contraction This observation shows that the uterine wall above the largest circumference of the foetal head loses contact with the foetal head during contractions

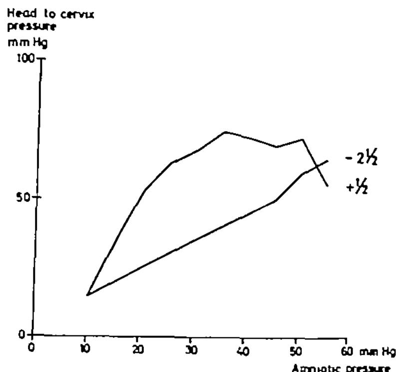


Fig. 3 Graph of the head to cervix pressure during a contraction. +0.5 = 0.5 cm above the largest circumference of the foetal head. 2.5 = 2.5 cm below this level.

This observation may have an application in some cases during vacuum extraction when the cervix is not fully dilated.

Water was also poured into the tube from above and the height of the column of water was measured from the top of the sphere by means of a scale outside the rubber tube (Fig. 2). At and below the equator of the sphere an increase in pressure between sphere and rubber tube corresponded to that seen in the traction study. Above the equator a rapid increase of pressure between sphere and rubber tube occurred initially but with an increase in the height of the column of water a decrease in the sphere–rubber tube resulted. This was because the rubber tube above the sphere distended and the area of contact with the sphere decreased. This corresponds with conditions pertaining in normal cervical dilatation.

During labour the head to cervix pressure is greatest at the

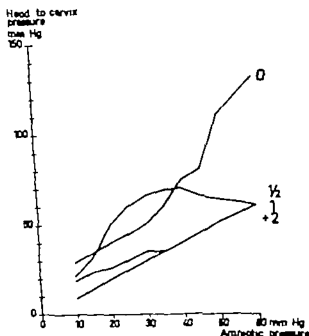


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Table I. *Normal Labour*

Case	Parity	Rupt. of Memb.	Birth Weight (g)	Occipito-bregmatic circ. (cm)	F (kp)	N (kp)	f
1	I	+	3560	33.5	4.8	25.1	0.19
2	I	+	3090	32	5.0	24 7	0.21
3	I	0	3110	34	10.4	57 4	0.19
4	II	+	3810	35	7 0	33.7	0.21
5	II	0	3740	34	7.6	35.2	0.21

F = Friction N = Resistance of cervical tissue f = coefficient of friction.
Estimated in patients with cervical dilatation of 5 cm.

Table II *Spasm of the Lower Uterine Segment*

Case	Parity	Rupt. of Memb.	Birth Weight (g)	Occipito-bregmatic circ. (cm)	F (kp)	N (kp)	f×N (kp)	D (kp)
1	I	+	3650	37.5	14 4	96.5	19.3	4.9
2	II	0	4200	36	7 8	60.5	12.1	4.3

F = Friction N = Resistance of cervical tissue f = coefficient of friction.
D = Difference
Estimated in patients with cervical dilatation of 5 cm.

In some cases of normal labour the coefficient of friction has been calculated (Table I) We found that this varied between 0 19 and 0.21 (average 0 2)

In two cases of spasm of the lower uterine segment the driving force was estimated from amniotic fluid pressure and the coefficient of friction. We found in these cases that the driving force was 4.9 and 4 3 kp respectively smaller than the calculated friction (Table II) In such cases there is probably no normal gliding movement between the foetal head and the uterine wall. At maximum contraction the uterine wall is in addition in contact with the foetal head above the largest circumference and this results in increased friction This friction of the foetal head

explains the delayed or arrested cervical dilatation in such cases. If one tries to dilate the cervix using the vacuum extractor in cases of spasm of the uterine segment the friction increases still more above the largest circumference as in the model study by traction and cervical dilatation is impossible. Sometimes the same effect may occur in normal labour if the normal increase in width of the uterus upwards is not present. A similar situation may arise in cases of oligohydramnios especially when traction is applied between contractions.

SUMMARY

In vertex presentation the friction between the foetal head and the uterine wall has been studied by means of intrauterine tocometry. The results have been compared with those in model studies using a wooden sphere in a rubber tube. In normal contractions the uterine wall above the largest circumference of the foetal head (the equator of the foetal head) loses contact with the foetal head as the rubber tube above the equator of the sphere when the rubber tube is filled by a column of water. The friction decreases above the equator in both cases. In lower uterine spasm and by traction of the sphere by weights the friction increases above the equator during contraction. This observation may explain the poor effect by using vacuum extractor in lower uterine spasm and in oligohydramnios.

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Case	Parity	Rupt. of Memb.	Birth Weight (g)	Occipito-bregmatic circ. (cm)	F (kp)	N (kp)	f×N (kp)	D (kp)
1	I	+	3850	37.5	14.4	96.5	19.3	4.9
2	II	0	4200	36	7.8	60.5	12.1	4.3

F = Friction N = Resistance of cervical tissue f = coefficient of friction.
D = Difference
Estimated in patients with cervical dilatation of 5 cm.

In some cases of normal labour the coefficient of friction has been calculated (Table I). We found that this varied between 0.19 and 0.21 (average 0.2).

In two cases of spasm of the lower uterine segment the driving force was estimated from amniotic fluid pressure and the coefficient of friction. We found in these cases that the driving force was 4.9 and 4.3 kp respectively smaller than the calculated friction (Table II). In such cases there is probably no normal gliding movement between the foetal head and the uterine wall. At maximum contraction the uterine wall is in addition in contact with the foetal head above the largest circumference and this results in increased friction. This friction of the foetal head

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Ingolfsson, Arni, Acta obst. et gynec. scandinav. 48, 456 1969

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BROW PRESENTATIONS

BY

ARNI INGOLFSSON

The present paper concerns cases of brow presentation and related deflexed attitudes (between brow and face and brow and median vertex) that occurred in the Department of Obstetrics and Gynaecology in Lund during the years 1932 to 1967 During this period 88,988 deliveries took place at the department (Table I) The frequency of brow presentation was 1 15 ‰ *i.e* about one in 860 and during 1900–1931 the incidence was one in 800 deliveries (*Sjövall* 1933) The frequency has obviously been quite constant during the past 67 years

The frequency of brow presentation in our department is somewhat higher than in most other reports. The department receives all parturients in the area served by the hospital except those who are delivered in the small maternity units in nearby hospitals. Since 1958 there have been practically no home deliveries at all in the country The material presented here comprises 91 cases of brow presentation, 6 of brow face presentation and 6 of brow median vertex presentation.

Aetiological Factors

The factors leading to brow presentation are considered to be the same as those causing deflexion attitudes in general. The question is why the head adopts precisely this intermediate attitude between vertex and face presentation.

Too early drainage of the amniotic fluid *i e* rupture of the membranes before labour has begun has previously been con-

sidered an important cause of deflexion attitudes. It is just as plausible, however, that too early drainage of amniotic fluid is caused by the deflexion, the foetal head being less well adapted in the pelvis. In 27 cases—26 per cent—early rupture of the membranes occurred.

Disproportion between the pelvis and the foetal head can cause deflexion. Disproportion was considered to be present in 16 cases (15.5 per cent), in 3 cases owing to a too big child.

Ample space between the foetal head and the pelvic walls may also result in brow presentation. Six infants (5.8 per cent) were premature, with a birth weight of less than 2500 g.

It is not unusual for twins to be born with the head more or less deflexed. Between 1932 and 1967, 1176 twins were delivered in our department. Of these, 7 (0.6 per cent) were born in brow presentation.

Multiparity has been thought to be one of the most important causes of deflexion attitudes, due to slackness in the abdominal muscles, possibly in association with uterine inertia. In this series (Table 1) 51 of the brow presentations occurred in *primiparae* (1.35 ‰) and 52 (1.02 ‰) in multiparae. Brow presentation were thus somewhat more common in primiparae.

Borell and *Fernström* (1960) believe that the reason the head persists in brow presentation probably is that the mouth of the foetus is open and the foetal chin is pressed against the chest. *Borell* and *Fernström* also describe a method to correct the brow presentation to face presentation by closing the mouth of the foetus. In our series in one instance X-ray showed presentation with the mouth of the foetus open.

Table 1. *Frequency of Brow Presentation in Primiparae and Multiparae* (1932–1967)

Gravida	Deliveries	Brow Presentation	Per 1000 Deliveries
Primi-	37,902	51	1.35
Multi-	51,066	52	1.02
Total	88,968	103	1.15

Ingolfsson, Arni, Acta obst. et gynec. scandinav. 48, 486, 1969

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Aetiological Factors

The factors leading to brow presentation are considered to be the same as those causing deflexion attitudes in general. The question is why the head adopts precisely this intermediate attitude between vertex and face presentation.

Too early drainage of the amniotic fluid, *i e* rupture of the membranes before labour has begun has previously been con

Table IV *Duration of Labour in Hours (Average)*

Gravida	Vaginal	Caesarean Section
Primi-	27 2/3	18 1/3
Multi-	14	7 1/2

Table V *Prolonged Labour in Brow Presentation*

Gravida	Prolonged Labour	Per 100 Deliveries
Primi-	22	43
Multi-	21	40
Total	43	41.5

Mor than 18 hours in primiparae and 12 hours in multiparae.

Clinical Course

According to various reports the delivery in brow presentation is spontaneous in between 12 and 44 per cent. In the present series 46 cases (45 per cent) were delivered spontaneously

As can be seen in Tables IV and V prolonged labour is very common. In the tables, an upper limit is set at 18 hours as normal for primigravidae and 12 hours for multigravidae (i.e. a woman with earlier labour) Prolonged labour occurred in 43 per cent of primigravidae and in 40 per cent of multigravidae Of all deliveries 21 (20 per cent) took 30 hours or more. In eight cases, delivery lasted for five hours or less. All eight were multigravidae except one (premature delivery 1350 g)

From this it can be concluded that brow presentation labours of short duration are very unusual among primigravidae and that prolonged labours are very common in both primigravidae and multigravidae The reason for the prolonged labour is most often dystocia To a lesser extent uterine intertia, also plays a part. 27 cases showed signs of inertia, 16 being recorded as primary and 11 as secondary

Cranial anomalies in the foetus can produce deflexion of the foetal head. Our series contains two instances of anencephalic monsters.

In 22 cases, the umbilical cord was twisted once or several times round the neck of the child. There is also one instance of polyhydramnios, one of prolapse of the cord, and one of placenta praevia. In two instances, there were fibroids in the lower part of the uterus.

In four cases external version of breech presentation had been made during pregnancy and in two from transverse lie (5.8 per cent) Consequently it seems that external version may cause deflexion of the foetal head.

Diagnosis

Table II shows the methods used for diagnosis and Table III at the stage of delivery when the diagnosis was made. The diagnosis was made late in a large percentage of the cases in about 47 per cent, not until delivery This agrees with other reports. In the spontaneous deliveries the diagnosis of brow presentation was not made until delivery in 78 per cent.

Table II. *Brow Presentation and Methods of Making the Diagnosis*

Method	Number of Patients
Rectal examination	7
Vaginal examination	22
X-ray examination	26
At delivery	48
Total	103

Table III. *The Stage of Labour when the Diagnosis of Brow Presentation Was Made*

Stage I	32
Stage II	23
At delivery	48
Total	103

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More than 18 hours in primiparae and 12 hours in multiparae.

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B. *Forceps or vacuum extraction* 26 patients were delivered by forceps or vacuum extraction.

Seventeen of the children were in fronto-anterior position when the intervention was begun. In two the foetal head was rotated to occipito-anterior position. In one case correction to face presentation was attempted but failed.

In two cases the heads were in fronto-transverse position and both were rotated to fronto-anterior position.

Four children were in fronto-posterior position. Two were rotated to fronto-anterior position, the other two were corrected to occipito-posterior position before extraction.

In three instances the position was not recorded with sufficient exactness in the case notes.

C. *Craniotomy and cranioclasty* Craniotomy and cranioclasty were performed on dead foetuses in three cases.

The first, from 1939 concerned a primigravida with total of 93 hours labour pains and fever The last 40 hours prior to delivery no foetal heart sounds were audible The foetus was extracted in fronto-anterior position.

The second, from 1957 concerned primigravida with labour pains for 20 hours The foetal heart was no longer audible about 4 hours before delivery The position was fronto-anterior

The third, f om 1967 concerned a gravida IV with labour pains for about 8 hours On admission the labour pains wer moderate. Immediately after the foetal heart sounds were established as normal but ten minutes later no longer were audible At vaginal examination fronto-posterior position was diagnosed At full dilatation attempts wer made to correct the position of the head, but when this failed, craniotomy and cranioclasty were per formed

D *Podalic version and extraction* This intervention was performed in two instances.

The first, in 1947 concerned gravida III with labour pains for 18.5 hours. The exact position of the foetal head is not stated in the case ecord Before version efforts to flex the head as well as attempts to deflex the head to fac presentation and apply forceps and make extraction had failed In spite of this living child weighing 4150 g was delivered by ersion and extrac tion

The second case in 1953 was gravida III with labour pains for 9.75 hours The foetal head was in fronto-transverse position. Attempts to rotate the head failed. Attempts to flex the head and to apply forceps in order

Table VI. *Position of the Foetal Head at Spontaneous and Operative Delivery*

Partus	Fronto-anterior	Fronto-transverse	Fronto-posterior	Undetermined	Total Number	Total Per Cent
Spontaneous	42	2	2		46	45
Forceps or *VE*	17	2	4	3	26	25
Caesarean section	7	4	4	10	25	24
Craniotomy and Cranioclasty	2		1		3	3
Version and extraction after failed forceps		1		1	2	2
Hysterectomy on account of uterine rupture				1	1	1
Total	68	9	11	15	103	100

Of those delivered in fronto-posterior position, one, weighing 3700 g, was the child of a secundigravida. X-ray pelvimetry a few days after delivery showed a true conjugate of 12.5 cm.

The mother of the other child was a primigravida. This child also weighed 3700 g. It was born in a presentation intermediate between fronto-posterior and median vertex presentation. Both the children were living.

Mode of Delivery

A. *Spontaneous deliveries.* Delivery occurred spontaneously in 46 cases. 42 of the children were born with the head in the fronto-anterior position, two in the fronto-transverse and two in the fronto-posterior position.

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The mother of the other child was a primigravida. This child also weighed 3700 g. It was born in a presentation intermediate between fronto-posterior and median vertex presentation. Both the children were living.

Cases of spontaneous delivery in fronto-posterior presentation have been reported previously (*Johansson* 1945, *Ingerslev* 1951) and it should be stressed that this can occur also when the child is rather big. Most of those children were also living.

Table VII. *Indications for Caesarean Section*

Indication	Number of Patients	Percentage
Disproportion (pelvis/head)	14	56
Prolonged labour + arrest	5	20
Other causes (Placenta praevia, elderly primigravidae etc.)	6	24
Total	25	100

Table VIII. *Summary of Spontaneous Deliveries and Mortality*

Spontaneous delivery	46 patients
Discharged living	38 infants
Dead at delivery	8 infants
Of these,	
Anencephalus	2 infants
Erythroblastosis	2 infants
Mongolism	1 infant
Corrected mortality	3 infants (7 per cent)

Table IX. *Summary of Forceps and VE Operations and Perinatal Mortality*

Forceps and VE operations	26 patients
Discharged living	21 infants
Dead at delivery	5 infants
Of these,	
Premature (1350 g) (30th week)	1 infant
Corrected mortality	4 infants (16 per cent)

Table X. *Brow Presentations. Frequency of Caesarean Sections and Perinatal Mortality at Different Periods*

Period	Number of Deliveries	Frequency of Caesarean Section (per cent)	Corrected Perinatal Mortality (per cent)
1900–1931 (Sjövall 1933)	46	2	21
1932–1950	53	8	18
1951–1967	50	45	6
(1958–1967)	30	54	3

to extract the child failed. After podalic version a dead child weighing 4500 g was extracted.

In the series there is also a case of uterine rupture from the year 1944

Gravida VI, 38 years of age. Five previous normal deliveries, all infants weighing between 4000 and 5000 g. Labour pains for 101 hours. X-ray on the second day of labour showed deflexion attitude between median vertex and brow presentation. Approximately 24 hours before delivery no foetal sounds were audible. The patient suddenly felt pain over the symphysis and became pale and ill. Laparotomy was immediately carried out. The rupture of the uterus included also the upper part of the vagina. A subtotal hysterectomy was carried out as well as suture of the vaginal rupture. The patient died at the end of the operation. The foetus was dead, it weighed 5200 g.

E *Caesarean section. Von Khreninger-Guggenberger* suggested (1930) that Caesarean section should be resorted to more often in brow presentations.

The *frequency of Caesarean section in cases of brow presentation* has, according to various authors risen successively. Whereas *Sjövall* (1933) reported a frequency of only two per cent for 46 cases during the years 1900–1931 and *Ingerslev* four per cent for 200 cases (1951) *Magid* and *Gillespie* (1957) have a figure of 37.5 per cent and *Jacobson* and *Johnsson* (1962) 18 per cent.

Dividing our material into two periods (Table X) gives a frequency of Caesarean section of 8 per cent before 1950 and 45 per cent after. During the past ten years, Caesarean section was undertaken in no less than 15 cases (over 50 per cent). The tendency at our department is to perform Caesarean section more and more often for brow presentation. Table VII shows the indications for Caesarean section. Of the Caesarean section cases one was a gravida II with brow presentation on both occasions the patient was operated upon by Caesarean section in both instances.

Perinatal Mortality

In the literature figures for infant mortality vary between six per cent and 25 per cent. In our series the corrected *mortality* is 12 cases. This is a high figure compared with the general peri

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in 45 per cent. During the past ten years Caesarean section was performed in over 50 per cent and the corrected perinatal mortality during this period was three per cent (only the craniotomy case from 1967) It is thus obvious that the prognosis for the child in brow presentation has successively improved with the increase in the Caesarean section rate (Table X)

However the question remains whether as soon as a brow presentation is diagnosed Caesarean section should always be resorted to or expectancy might be permitted.

It should be kept in mind that brow presentation can change spontaneously to face–or even vertex presentation during the course of labour

Forty-six of the deliveries in brow presentation were spontaneous and most of those who required termination by forceps or vacuum extraction were successful. On the other hand those spontaneous deliveries, forceps and vacuum extractions had a rather high perinatal mortality seven per cent and 16 per cent respectively The conclusion seems therefore to be that expectancy might be permitted only under very favourable conditions such as a roomy pelvis, a not too big foetus, excellent uterine contractions and good progress of labour As soon as labour does not progress or even moderate complications occur Caesarean section should be undertaken. High and mid forceps operations and especially podalic version and extraction should be avoided, whereas outlet forceps or vacuum extraction in brow presentation can be made Simple methods of manual correction such as that described by *Borell* and *Fernström* should also be considered in suitable cases.

SUMMARY

The 103 cases of brow presentation which occurred at the Department of Obstetrics and Gynaecology in Lund during the years 1932–1967 are reviewed, especially regarding the perinatal mortality

Spontaneous deliveries in brow presentation occurred in 45 per cent forceps or vacuum extraction in 25 per cent and Caesarean section in 24 per cent.

Table XI. *Perinatal Mortality Before and after 1950*

Up to and Including 1950	1950 Onwards
Total 53 patients	Total 50 patients
Discharged living 41 infants (77 per cent)	Discharged living 44 infants (88 per cent)
Perinatal deaths 12 (23 per cent)	Perinatal deaths 6 (12 per cent)
Of the 12,	Of the 6
1 mongoloid	1 erythroblastosis (intrauterine death)
1 anencephalus	1 anencephalus
1 erythroblastosis	1 premature (1350 g) (30th week)
Corrected mortality 9 infants (18 per cent)	Corrected mortality 3 infants (6 per cent)

natal mortality in the department during the years 1945–1967 At the beginning of this period it was 2.7 per cent and has slowly fallen to 1.8 per cent in 1967 This demonstrates how unfavourable brow presentation is for the foetus. The spontaneous deliveries in brow presentation (Table VIII) show a corrected mortality of 7 per cent. If however the two craniotomy cases and the uterine rupture case—all of which were caused by unduly prolonged expectancy—are added to the spontaneous deliveries a total of six dead children (approximately 13 per cent) is obtained.

In the forceps and vacuum extraction operations (Table IX) the corrected mortality is 16 per cent.

Podalic version and extraction was carried out in two cases after failed attempts at correction and forceps extraction. One of the children died.

In the Caesarean section cases there was no foetal mortality

Discussion

The entire series is divided as shown in Table XI into two periods. It can be seen that the perinatal mortality before 1950 was 18 per cent and after six per cent Before 1950 Caesarean section was undertaken in eight per cent of the cases, after 1950

Lundvall, F and Stakemann, G. Acta obst. et gynec. scandinav. 48, 497 1968
From the Department A of Obstetrics and Gynaecology (Professor Dyre Trolle and Borge Sorensen M.D.) Rigshospitalet Copenhagen Denmark

THE URINARY EXCRETION OF OESTRIOL IN PREGNANCIES WITH RH IMMUNISATION

BY

F. LUNDVALL AND G. STAKEMANN

Estimation of the urinary excretion of oestriol has received increasing interest from obstetricians. It seems to reflect the condition of the foetus and in this way it can be used as a guide in the treatment of various pathological conditions. Low or declining values appear to indicate that danger to the foetus is present and extremely low values indicate foetal death.

In pregnancies complicated by Rh-immunisation reports are somewhat conflicting. In such cases *Taylor et al.* (1963) found a higher output of oestriol than in normal pregnancies. In contrast *Klopper* and *Stephenson* (1966) in 49 cases, found values within the normal range even in severely sensitized cases. *Banerjea* (1962) found high values in a moderately sensitized patient. In another where the foetus died in utero he found low values. *Schindler et al.* (1967) found that the concentration of oestriol in the amniotic fluid reflected the condition of the foetus better than either the concentration in plasma or the urinary excretion.

The present investigation is a retrospective analysis of the correlation between the foetal status and the maternal urinary excretion of oestriol in a rather large series of patients.

Method

All hormone estimations were carried out at the hormone department of the Seruminstitute using the method developed by F andsen (1963)

Podalic version and extraction after failed forceps was made in two cases.

Craniotomy was required in three cases.

One mother died. This occurred during performance of a subtotal hysterectomy for rupture of the uterus.

The corrected total perinatal mortality was 12.4 per cent. It has successively fallen from 18 per cent during 1932–1950 to 6 per cent after 1950 and during the last ten years to 3 per cent along with an increase in the Caesarean section rate from 8 per cent to 45 per cent and over 50 per cent respectively.

In very favourable cases of brow presentation spontaneous delivery or easy outlet extraction can be considered, in others Caesarean section should be undertaken.

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Material

At the Rigshospitalet obstetrical department A 156 consecutive patients with Rh immunisation were investigated. Only a few cases had to be excluded because the patients were uncertain of the date of their last menstrual period. Values obtained after obvious foetal death have been excluded. The material has been divided into two groups one where Coombs test on cord blood indicated Rh affected babies and another where no such affection could be demonstrated The first group consisted of 122 patients the second of 34

Twenty-four hours urine specimens were collected at weekly intervals during the last month before calculated term, and in many cases samples were also collected eight and twelve weeks before term. Treatment of the patients was in no way influenced by the results of the hormone analyses.

The obstetrical management was based on the estimated degree of immunisation as judged by the results of repeated indirect Coombs tests during pregnancy and by the past obstetrical history Intrauterine transfusions were not used during the period under review but in the more severely affected cases labour was induced or Caesarean section carried out when the foetal weight was estimated to be 2800 g *i.e* two weeks before term. Fifty-nine women had labour induced and five had Caesarean section.

The degree of Rh affection of the babies as judged from the concentration of haemoglobin in the cord blood is shown in Table I

Table I. *Haemoglobin-concentration in Cord Blood*

g/100 ml	Number of Babies	
	Rh affected	Rh unaffected
>17.8	13 (11)	12 (35)
<17.8–14 8	37 (30)	18 (53)
<14.8–11 8	46 (38)	4 (12)
<11 8	26 (21)	0 (0)

Figures in brackets show percentages

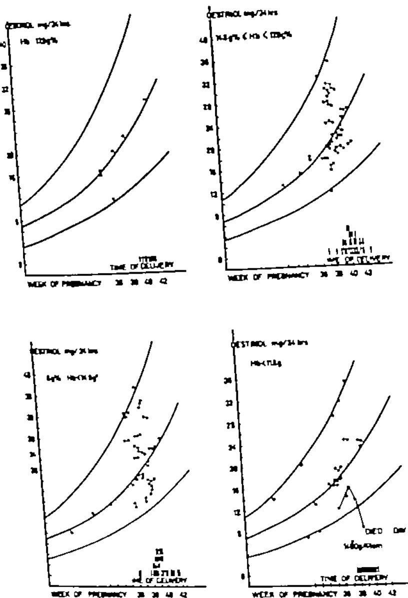


Fig. 1. Excretion of oestriol in 122 Rh-immunized patients delivered of affected babies.

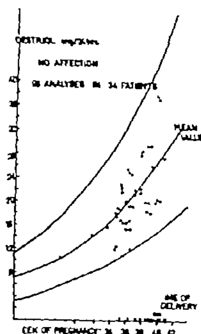
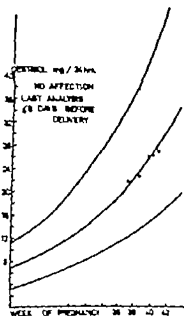


Fig. 2. Excretion of oestriol in 34 Rh-immunized patients delivered of unaffected babies



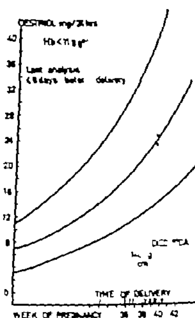


Fig. 3. The oestriol excretion estimated during the last week before delivery in patients delivered of unaffected and of severely affected babies.

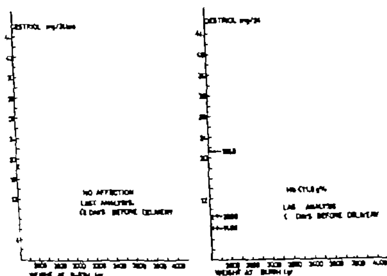


Fig 4 The values of Fig 3 m elation to birth weight

Results

Figure 1 shows all the values in the series according to the concentration of haemoglobin in cord blood. The lines indicate the range and average excretion of oestriol in normal pregnancies (from F andsen and *Stakemann* 1963) The oestriol output in Rh-immunized patients shows a distribution similar to that of normal pregnancies even in cases where the baby was severely affected. For comparison is shown the excretion of patients where the babies were unaffected (Fig. 2)

It might be that a difference in the hormone excretion between mild and severe cases was present only shortly before term. Figure 3 shows a comparison of the hormone excretion estimated less than one week before delivery in patients with unaffected babies and those with severely affected babies. No significant difference can be found.

It has been shown that a correlation exists between the weight of the baby and the amount of oestriol excreted (*Frandsen* and

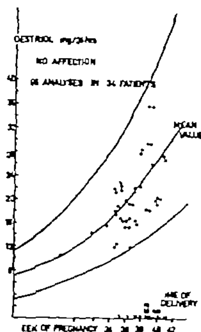
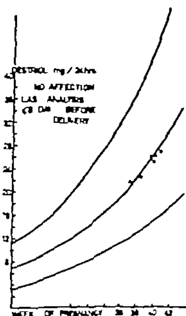


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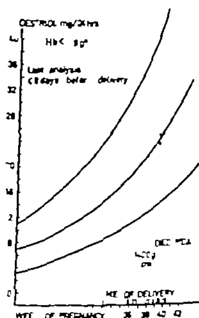


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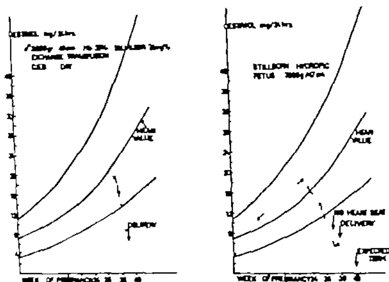


Fig. 6 Oestriol excretion in two patients in which the life of the foetus was severely jeopardized.

Scakemann 1960 *Coyle* and *Brown* 1963) The values from Figure 3 are therefore shown in relation to the birth weight of the baby in Figure 4

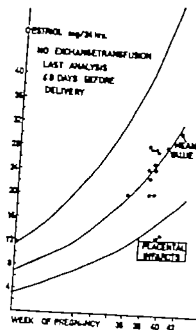
Again no difference can be demonstrated between patients with unaffected and patients with severely affected babies.

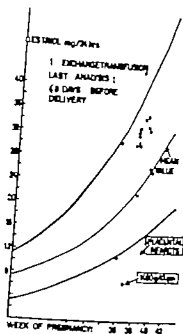
Another parameter for evaluation of the degree of erythroblastosis in the baby is the number of exchange transfusions necessary

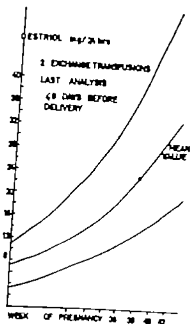
In Fig. 5 the series has been analysed according to the number of exchange transfusions given

The results show no significant difference in the oestriol excretion just before delivery in cases needing none, one, two or three or more transfusions.

Although Rh-immunisation *per se* apparently does not influence the maternal urinary excretion of oestriol a low output can be demonstrated in cases where the life of the foetus is severely jeopardized. Fig. 6 shows two such cases.







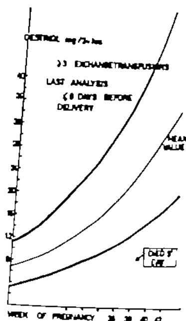


Fig. 5 Comparison of oestriol excretion in relation to the number of exchange transfusions needed.

Nielsen, Niels Chr. Acta obst. et gynec. scandinav. 48, 305, [illegible]
From the University Maternity Hospital (Professor M. Ingerslev) Aarhus Denmark

COAGULATION AND FIBRINOLYSIS IN PREMATURELY DELIVERED MOTHERS AND THEIR PREMATURE INFANTS

By

NIELS CHR. NIELSEN

In a number of recent communications it has been confirmed that the content of several coagulation factors in the blood increases as pregnancy advances. Fibrinolysis during pregnancy has also been thoroughly studied.

Coagulation and fibrinolysis in premature babies have been studied in a number of publications. However, it is difficult to compare the studies, because the blood samples were taken at different times after delivery and because different techniques were used. Moreover, the term prematurity is usually based only upon the birth weight (under 2500 g) without paying regard to gestational age.

The objekt of the present work was to study coagulation and fibrinolysis in a well-defined group of infants born more than four weeks before term in whom the blood samples were removed under standardized conditions. The results will be compared with those obtained by analyses on maternal blood.

This material allows subsequent comparison with similar findings in premature babies from pregnancies complicated by e.g. to rhesus iso-immunization, pre-eclampsia, and abruptio placentae.

Previous Investigations

In the present section reference will be made only to publications in which the analyses, in premature babies, have been based

Discussion

Taylor et al (1963) found high excretion values of oestriol in pregnancies complicated only by Rh-immunisation. If this is correct it would be necessary in such cases to raise the upper limit of the oestriol output supposed to indicate that the foetus is stressed. However both the present series and that of *Klopper* and *Stephenson* (1966) show that the oestriol excretion in such cases is within the range found in completely normal pregnancies, and consequently the limits of the "danger zone need no alterations.

SUMMARY

The excretion of oestriol in 156 cases of apparent Rh-immunisation was found to be in the same range as that of normal pregnancies

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In a number of recent communications it has been confirmed that the content of several coagulation factors in the blood increases as pregnancy advances. Fibrinolysis during pregnancy has also been thoroughly studied.

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The objekt of the present work was to study coagulation and fibrinolysis in a well-defined group of infants born more than four weeks before term in whom the blood samples were removed under standardized conditions. The results will be compared with those obtained by analyses on maternal blood.

This material allows subsequent comparison with similar findings in premature babies from pregnancies complicated by *e.g.* to rhesus iso-immunization, pre-eclampsia and abruptio placentae.

Previous Investigations

In the present section reference will be made only to publications in which the analyses, in premature babies, have been based

upon cord blood, since a number of changes occur very soon after birth in coagulation (*Beller* 1955 *Vest* and *Meier* 1957 *Hansen* 1960 and others) as well as in fibrinolysis (*Engström* and *Kager* 1964)

First Phase of Coagulation.

Platelets In two comprehensive studies *Talbert* and *Langdell* (1964) and *Nilsson* and *Kullander* (1967) found that the maternal platelet count did not change during the last trimester

In blood from the umbilical vein *Hansen* (1960) found subnormal platelet counts average 168 000/mml, in a study of 47 premature infants but he did not state any control values from mature infants.

Factor VIII (antihaemophilic factor AHF) The reported findings have varied somewhat but recent investigations have shown the content to increase through pregnancy (*Strauss* and *Diamond* 1963 *Kasper et al.* 1964 *Nilsson* and *Kullander* 1967 and others)

Factor VIII activity is reduced in the cord blood of premature but not in mature infants. *Künzer* (1959) for instance, found an average of only 60 per cent of normal although a number of the values were in the normal range

Factor IX (Christmas factor) *Ratnoff* and *Holland* 1959 and *Kasper et al* 1964 have reported an increased level in the blood in the 3rd trimester of pregnancy while *Nilsson* and *Kullander* (1967) and others were unable to demonstrate such an increase.

Künzer (1959) has demonstrated a marked reduction of factor IX in the cord blood of all premature babies so studied.

The prolonged recalcification time reported by *Künzer* (1959) is in keeping with the reduced content of factors VIII and IX.

Second Phase of Coagulation

Factor II (prothrombin) *Beller* (1957) and *Fresh et al* (1959) found an increase in maternal blood during the latter part of pregnancy while *Kasper et al* (1964) and *Talbert* and *Langdell* (1964) found only slight or no changes

Table I. Results of Coagulation and Fibrinolysis Studies in Normal Women Immediately ... Delivery

Tests, see Methods	Normal Women Immediately after Delivery (Weight of Newborns)					
	>2500 g		>2000 g<2500 g		≦2000	
	x̄ and n	Range est. SD	x̄ and n	Range est. SD	x̄ and n	Range est. SD
Platelets	207	67–351	231	139–448	215	172 25
(Thousands per cu. mm.)	n=20	±76	n=10	±90	n=10	±27
Proaccelerin	110	80–190	101	75–125	110	87 13
(per cent)	n=20	+31	n=10	±17	n=10	±14
Prothrombin time	17	16–20	18	15–20	18	17 20
(seconds)	n=20	±0.9	n=10	±1.7	n=10	±0.8
P and P	206	120–380	178	115–245	166	140–21
(per cent)	n=20	±68	n=10	±44	=10	±23
Partial thromboplastin	73	66–79	75	67–85	75	71–81
time (seconds)	n=20	+3.6	n=10	±5	n=10	+3.
Thrombin time	8	5–10	6	5–8	7	5–8
(seconds)	=20	±1.1	n=10	±1	n=10	±1.
Fibrinogen	508	320–985	515	440–650	519	390–73
(mg per 100 ml)	=20	±154	n=10	±57	n=10	±10
Haematocrit	42	36–48	41	35–55	42	38–46
(per cent)	=20	±3.2	n=10	±1.8	n=10	±2.7
Standard fibrin plates	0	0	0	0	0	0
Untreated plasma						
(sq. mm.)	=20	±0	=10	±0	n=10	±0
Standard fibrin plates	39	0–105	30	0–93	21	0–49
Euglobulins (sq. mm.)	=20	±25	=10	±35	n=10	±16
Heated fibrin plates	0	0	0	0	0	0
Untreated plasma						
(sq. mm.)	=20	±0	n=10	±0	n=10	±0
Heated fibrin plates	27	0–49	21	0–40	14	4–49
Euglobulins (sq. mm.)	=20	±14	=10	±14	n=10	±14
Plasminogen (μg Cu-Tyrosine per ml)	173	103–234	169	126–194	175	125–23
	=20	±25	=10	±18	=10	±2

x̄ = arithmetic average, = number of estimations

Range = range of individual results ( ) est. SD = estimated standard deviation

$$= \pm \sqrt{\frac{\Sigma(\ - \bar{x})^2}{n-1}}$$

In previous studies on the relation of prothrombin levels to the maturity of the baby some authors have found lower and others higher values in premature than in mature infants but invariably the concentration has been reduced compared with adults. For reviews of the earlier literature the reader is referred to *Dyggve* 1952 and *Larsen* 1952. A large series, submitted by *Forgács et al.* (1962) who studied cord blood from 100 premature infants of birth weights from 750 to 1500 g compared with 104 full term infants clearly showed that the prothrombin content is lowest in the former group No details are given concerning the composition of this group

Factor VII (proconvertin) *Alexander et al* (1956) *Beller* (1957) *Pechet* and *Alexander* (1961) and others have reported an increase in the maternal blood in the last trimester

In cord blood the proconvertin level is reduced (*Künzer* 1959 *Hansen 1960* and *Forgács* 1962) The last-mentioned team also demonstrated lower values in premature than in mature infants.

Factor X (Stuart Prower factor) An increased content has been demonstrated in the maternal blood during the last trimester (*Pechet* and *Alexander* 1961) while in the cord blood of premature babies it has been found to be reduced (*Künzer* and *Ströder* 1960)

Factor V (proaccelerin) A number of workers have found an unchanged factor V level throughout pregnancy (including *Alexander et al* 1956 *Kasper et al* 1964 *Talbert* and *Langdell* 1964 and *Nilsson* and *Kullander* 1967)

According to some reports factor V too is reduced in the blood of premature babies (*Künzer* 1959 and *Forgács et al.* 1962) while *Hansen* (1960) found a level above the normal range but he himself doubted whether this result was correct.

Third Phase of Coagulation

Factor I (fibrinogen) The blood level steadily increases through out pregnancy (*Fresh et al* (1959) *Gillman et al* (1959) *Kasper et al* (1964) *Talbert* and *Langdell* (1964) *Brakman* (1966) *Nilsson* and *Kullander* (1967) and others) However

Table II. *Results of Coagulation and Fibrinolysis Studies in the Newborn*

Tests, see Methods	Infants Immediately after Delivery Weight					
	>2500 g		>2000 g<2500 g		≤2000 g	
	*$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD
Platelets	323	66–571	307	174–625	294	200–384
(thousands per cu. mm.)	n=20	±147	n=10	±144	n=10	±63
Proconvertin	115	62–170	92	59–115	85	34–150
(per cent)	n=20	±34	n=10	±21	n=10	±32
Prothrombin time	19	17–22	19	17–23	2[illegible]	20–24
(seconds)	n=20	±1.5	n=10	±2.1	n=10	±2.3
P and P	72	30–115			38	23–56
(per cent)	n=20	±25			n=10	±11
P and P*	90	62–150	44	27–79		
(per cent)	n=20	±21	n=10	±16		
Partial thromboplastin	88	78–125	112	97–144	142	104–212
time (seconds)	n=20	±11	n=10	±15	n=10	±32
Thrombin time	13	10–15	10	7–14	14	9–24
(seconds)	n=20	±1.5	n=10	±2.3	n=10	±4.7
Fibrinogen	242	150–340	264	140–370	221	145–270
(mg per 100 ml)	n=20	±50	n=10	±67	n=10	±[illegible]
Haematocrit	55	48–63	56	47–65	56	49–69
(per cent)	n=20	±4.9	n=10	±5.6	n=10	±4.0
Standard fibrin plates Untreated plasma	86	16–230	38	0–54	31	25–70
(sq mm)	n=20	±56	n=10	±22	n=10	±20
Standard fibrin plates	390	251–658	211	25–394	210	56–360
Euglobulins (sq mm)	n=20	±119	n=10	±104	n=10	±100
Heated fibrin plates Untreated plasma	25	9–49	12	0–25	11	0–20
(sq mm)	n=20	±9.7	n=10	±8.3	n=10	±6.2
Heated fibrin plates	34	19–49	27	1–49	24	16–36
Euglobulins (sq mm)	n=20	±7.6	n=10	±12	n=10	±5.7
Plasminogen (μg Co-Tyrosine per ml)	67	37–108	33	17–46	37	8–65
	n=20	±19	n=10	±12	n=10	±17

See footnote to Table I

Prophylactic treatment of the mothers with vitamin K.

In previous studies on the relation of prothrombin levels to the maturity of the baby some authors have found lower and others higher values in premature than in mature infants, but invariably the concentration has been reduced compared with adults. For reviews of the earlier literature the reader is referred to *Dyggve* 1952 and *Larsen* 1952. A large series submitted by *Forgács et al* (1962) who studied cord blood from 100 premature infants of birth weights from 750 to 1500 g compared with 104 full term infants clearly showed that the prothrombin content is lowest in the former group No details are given concerning the composition of this group

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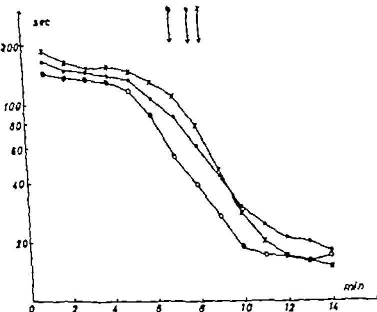


Fig. 1 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 20 normal women at term.
—— 15 normal adult women, two-stage TAT
——• 20 normal women, two-stage TAT immediately after delivery
—— 20 normal women, three-stage TAT immediately after delivery

or on serum or else the groups of babies studied were not directly comparable

A reduced fibrinolytic activity in premature as compared with mature infants has been demonstrated by *Arustowicz* (1966) and *Markarian et al.* (1967) who reported a reduced fibrinolytic activity in euglobulin plasma, by *Künzer* and *Ströder* (1961) and *Ambrus et al.* (1963) largely on the basis of an increased antiplasmin level in the premature babies. On the other hand, *Forgács et al* (1962) found the fibrinolytic activity to be in the same range in premature and in mature infants. Lastly *Chulla* and *Luraschi* (1954) reported that fibrinolytic activity was most marked in premature and stillborn children, but perhaps this result was due to anoxia.

Phillips and *Skrodelis* (1958) found the values to be the same in women during labour whether at or before term.

Forgács et al (1962) found a lower fibrinogen content in the cord blood of premature than of mature infants. *Phillips* and *Skrodelis* (1958) and *Panizza* (1962) arrived at the same result, but with such a marked range of values that there can hardly have been significant differences On the other hand, *Arustowicz* (1966) observed a significantly lower content in premature babies. However her technique of drawing blood was not ideal. The blood was drawn directly from the cord where it must have been in contact with the gelatinous substance with its high content of thromboplastin. *Markarian et al.* (1967) demonstrated a tendency to a higher fibrinogen content in premature babies, but six hours after birth there was practically no difference.

Haematocrit Most investigations have shown slightly decreasing haematocrit values towards term but with an increase shortly before. (For review of the literature *cf* *Nilsen* 1963)

The haematocrit level in cord blood from premature infants does not appear to have been investigated.

Fibrinolysis

Several studies have shown that fibrinolytic activity is reduced during the last months of pregnancy while it is normal or increased immediately after delivery The literature has been reviewed by *Nilsen* (1963) and by *Nilsson* and *Kullander* (1967) who also reported steadily increasing plasminogen content during pregnancy In delivered women at term *Samartzis et al.* (1960) *Skjødt* and *Albrechtsen* (1965) and others have also found an increased plasminogen activity whereas *Shaper et al.* (1965) and *Brakman* (1966) were unable to demonstrate any difference in the content between non-pregnant and pregnant women. Comparison of women in labour at term and women delivering healthy premature infants has shown no difference in fibrinolytic activity (*Markarian et al* (1967))

The reported data on fibrinolytic activity in the cord blood of premature babies have varied. This may be due to different techniques the analyses may have been performed on plasma

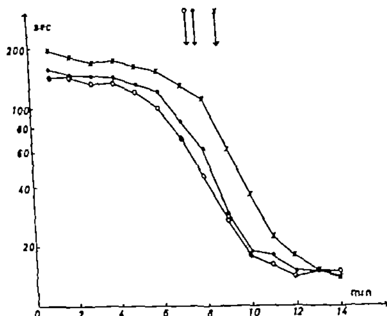


Fig 3 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women an average of six weeks before estimated date of confinement

—— 9 normal adult women two-stage TAT
—— 10 women, two-stage TAT immediately after delivery
—·— 10 women, three-stage TAT immediately after delivery

delivery and puerperium had otherwise been normal. The samples were removed within the first half-hour after delivery Prior to deli ery all the women (except one whose analysis did not differ from the others) had received prophylactic vitamin K, in a dosage as described previously (*Nielsen* 1969 a)

Blood samples were also drawn from these women s newborn infants within the first 20 minutes after birth. All the infants were healthy premature babies who behaved normally during their stay in hospital. The birth weights were below 2500 g, but above 2000 g, average 2270 g (range 2020–2440 g)

Group 2 Ten women and their newborn infants selected according to the same criteria as group 1 only with delivery ap-

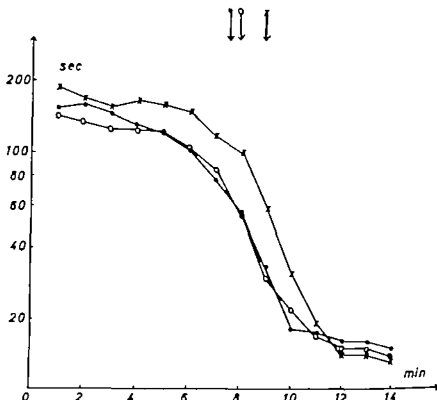


Fig. 2. Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women an average of five weeks before estimated date of confinement.

x ——— x 10 normal adult women two-stage TAT
• ——— • 10 women two-stage TAT immediately after delivery
o ——— o 10 women three-stage TAT immediately after delivery

Plasminogen activity on the other hand is reduced in the newborn particularly when they are premature (*Phillips* and *Skrodelis* 1958 *Quie* and *Wannamaker* 1960 *Samartzis et al.* 1960 *Pantl a* 1962 and *Ambrus et al.* 1963)

Material and Methods

The analyses were performed on blood samples from the following three groups

Group 1 Ten women who were delivered about 5 weeks (range 4–7 weeks) before estimated term but whose pregnancy

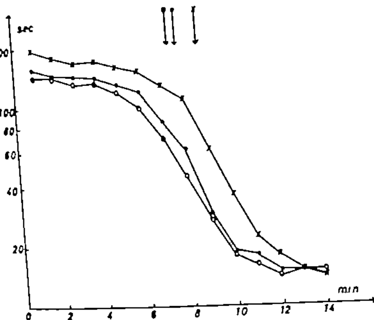


Fig 3 Thromboplastin activation curv of peripheral blood drawn immediately after delivery from 10 women an average of six weeks before estimated date of confinement.

—— 9 normal adult women, two-stage TAT
—— 10 women, two-stage TAT immediately after delivery
—— 10 women, three-stage TAT immediately after delivery

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Group 2 Ten women and their newborn infants selected according to the same criteria as group 1 only with delivery ap-

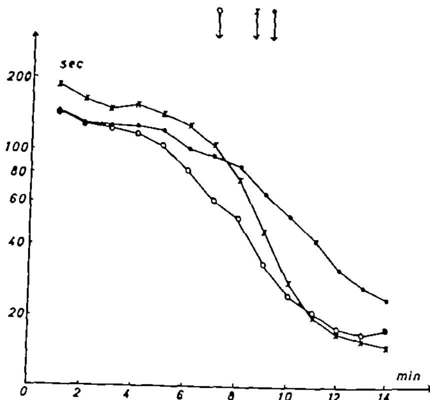


Fig. 4 Thromboplastin activation curve of venous cord blood from 20 normal mature infants.

x——x 15 normal adult women, two-stage TAT
●——● 20 normal mature infants two-stage TAT
o——o 20 normal mature infants three-stage TAT

prox. 6 weeks before estimated term (range 4–8 weeks) and birth weight ≤ 2000 g, average 1780 g (range 1440–2000 g) One infant, apparently normal at birth, died on the second day of life Autopsy revealed total pulmonary atelectasis with con gestion and incipient formation of hyaline membranes. The women in this group had not received prophylactic vitamin K.

Group 3 The control series comprised two groups One of 20 normal women delivered at term and their normal newborn infants. Vitamin K was administered and blood samples drawn just as in group 1 The other group also contained 20 normal women delivered at term and their normal newborn infants, but

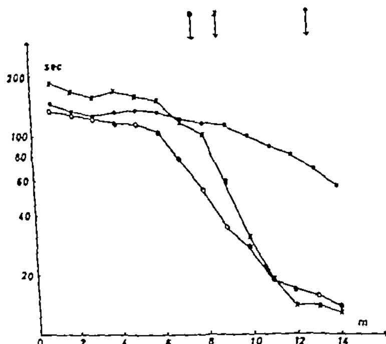


Fig. 5. Thromboplastin activation curve of venous cord blood from 10 normal premature infants, weight between 2500 g and 2000 g.

—— 10 normal adult women, two-stage TAT

•—— 10 normal premature infants, two-stage TAT

—— 10 normal premature infants three-stage TAT

in this group no vitamin K was administered. This control series has previously been described (*Nielsen* 1969 a)

The maternal blood samples were removed from an ante-cubital vein after application of light tourniquet. In the newborn the umbilical cord was incised close to the skin and disposable plastic catheter was inserted through the umbilical vein. The samples were used only if there was free flow of blood through the catheter. The technique of blood sampling and of the further treatment of the samples has been described in detail in previous publication. Determination of the recalcification time, thromboplastin activation test (TAT) two- and three-stage, partial thromboplastin time (PTT) thrombin time prothrombin time (Quick) factor V prothrombin-proconvertin (PP) fibrinogen content, and measurement of fibrinolytic activity in plasma and in iso-electrically precipitated plasma on untreated fibrin

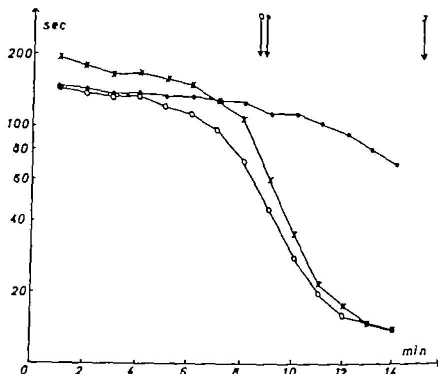


Fig. 6 Thromboplastin activation curve of venous cord blood from 10 normal premature infants weight below 2001 g.

x——x 9 normal adult women, two-stage TAT
•——• 10 normal premature infants two-stage TAT
o——o 10 normal premature infants three-stage TAT

plates (standard plates) and on heated fibrin plates as well as plasminogen, haematocrit, and platelet counts were also performed as already described (*Nielsen* 1969 a)

Results

The results of the *coagulation studies* are presented in Tables I and II those of TAT and recalcification times in Figs. 1 2, 3 4 5 and 6 For that part of the control group in which the mothers received prophylactic vitamin K all the results have been omitted with the exception of the babies PP since this is the only factor which is definitely influenced by vitamin K (*Nielsen* 1969 a)

In a comparison between group 1 (mothers with premature infants weighing between 2000 and 2500 g) and group 3 (nor-

al mothers and their infants) the following findings were made

A. *Peripheral maternal blood.* TAT two-stage showed no major fferences between the two groups except that t max was gnificantly shortened in the prematurely delivered women .02 > p > 0.01) TAT three-stage showed coagulation-activating mponents in the plasma from both groups possibly somewhat ss in the prematurely delivered women.

Apart from the shortened thrombin time ($p < 0.001$) in prematurely delivered women, there were no significant differences 1 coagulation findings.

B. *Cord blood.* TAT two-stage showed a distinctly flatter ourse in the premature babies with a significantly prolonged ecalcification time ($p < 0.001$) and t max ($0.01 > p > 0.001$) TAT three-stage was practically identical in both groups. The ndividual coagulation analyses demonstrate a considerably lower evel in premature babies. This applies for instance to PP ($p < 001$) and PTT ($p < 0.001$) The thrombin time was shortened ($p < 0.001$) whereas no change was found in fibrinogen content

The results of the *fibrinolysis studies* are also listed in Tables I and II. A comparison of group 1 with group 3 shows the following findings

A. *Peripheral maternal blood* No significant difference between the two groups.

B. *Cord blood* Significantly reduced fibrinolytic activity in premature babies, in untreated plasma on standard plates ($0.01 > p > 0.001$) and on heated fibrin plates ($0.01 > p > 0.001$) as well as in iso-electrically precipitated plasma on standard plates ($p < 0.001$) The plasminogen content too was reduced in the premature babies ($p < 0.001$)

In order to elucidate the influence of the degree of prematurity upon coagulation and fibrinolysis, group 1 (average birth weight 2270 g) was compared with group 2 (average birth weight 1780 g) The results were as follows

A *Peripheral maternal blood.* No significant changes in the parameters of coagulation or fibrinolysis.

B *Cord blood.* Within the group of infants whose birth weight averaged 1780 g there was a distinctly flatter curve for TAT

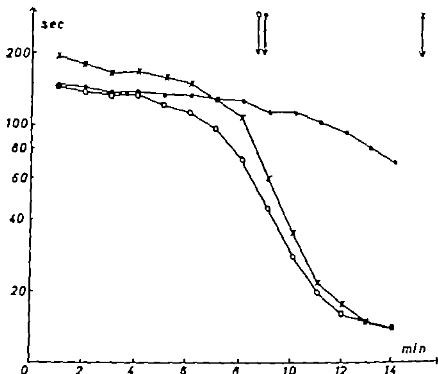


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x——x 9 normal adult women two-stage TAT
•——• 10 normal premature infants two-stage TAT
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Results

The results of the *coagulation studies* are presented in Tables I and II those of TAT and recalcification times in Figs. 1 2, 3 4 5 and 6. For that part of the control group in which the mothers received prophylactic vitamin K all the results have been omitted with the exception of the babies PP since this is the only factor which is definitely influenced by vitamin K (*Nielsen* 1969 a)

In a comparison between group 1 (mothers with premature infants weighing between 2000 and 2500 g) and group 3 (nor-

nal mothers and their infants) the following findings were made

A. *Peripheral maternal blood* TAT two-stage showed no major differences between the two groups except that t max was significantly shortened in the prematurely delivered women ($0.02 > p > 0.01$) TAT three-stage showed coagulation-activating components in the plasma from both groups, possibly somewhat less in the prematurely delivered women.

Apart from the shortened thrombin time ($p < 0.001$) in prematurely delivered women, there were no significant differences in coagulation findings.

B. *Cord blood* TAT two-stage showed a distinctly flatter course in the premature babies with a significantly prolonged recalcification time ($p < 0.001$) and t max ($0.01 > p > 0.001$) TAT three-stage was practically identical in both groups. The individual coagulation analyses demonstrate a considerably lower level in premature babies. This applies for instance to PP ($p < 0.01$) and PTT ($p < 0.001$) The thrombin time was shortened ($p < 0.001$) whereas no change was found in fibrinogen content

The results of the *fibrinolysis studies* are also listed in Tables I and II. A comparison of group 1 with group 3 shows the following findings

A. *Peripheral maternal blood.* No significant difference between the two groups

B. *Cord blood.* Significantly reduced fibrinolytic activity in premature babies, in untreated plasma on standard plates ($0.01 > p > 0.001$) and on heated fibrin plates ($0.01 > p > 0.001$) as well as in iso-electrically precipitated plasma on standard plates ($p < 0.001$) The plasminogen content too was reduced in the premature babies ($p < 0.001$)

In order to elucidate the influence of the degree of prematurity upon coagulation and fibrinolysis, group 1 (average birth weight 2270 g) was compared with group 2 (average birth weight 1780 g) The results were as follows

A *Peripheral maternal blood* No significant changes in the parameters of coagulation or fibrinolysis.

B *Cord blood* Within the group of infants whose birth weight averaged 1780 g there was a distinctly flatter curv for TAT

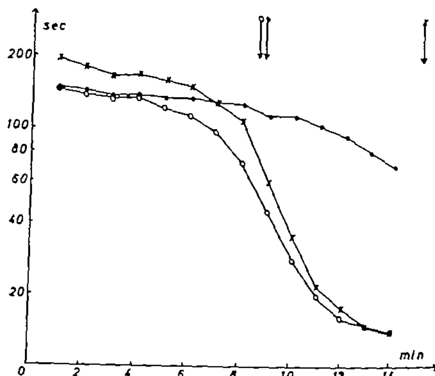


Fig. 6 Thromboplastin activation curve of venous cord blood from 10 normal premature infants weight below 2001 g.

x——x 9 normal adult women, two-stage TAT
●——● 10 normal premature infants two-stage TAT
o——o 10 normal premature infants three-stage TAT

plates (standard plates) and on heated fibrin plates, as well as plasminogen, haematocrit, and platelet counts were also performed as already described (*Nielsen* 1969 a)

Results

The results of the *coagulation studies* are presented in Tables I and II, those of TAT and recalcification times in Figs. 1 2, 3 4 5 and 6 For that part of the control group in which the mothers received prophylactic vitamin K all the results have been omitted with the exception of the babies PP since this is the only factor which is definitely influenced by vitamin K (*Nielsen* 1969 a)

In a comparison between group 1 (mothers with premature infants weighing between 2000 and 2500 g) and group 3 (nor-

with increasing prematurity Accordingly the results correspond with those in *Forgács et al.s* (1962) comprehensive study and appear to be in keeping with most of the findings reported in the literature.

Third phase of coagulation. As is apparent from the submitted literature, the fibrinogen content increases as pregnancy advances. In this material, too there was an increased content in women after delivery but this increase was of the same magnitude in women who were delivered at term as in those who went into labour about 6 weeks before the estimated date of confinement. Thus, the maximum fibrinogen content appears to be attained at a fairly early stage of the third trimester The thrombin time was also the same in all three groups of delivered women.

No difference was found in the content of fibrinogen in premature and mature infants, but the results are comparable to those already published. However the results for premature babies in the study of *Forgács et al.* (1962) were lower but as already mentioned their material consisted of infants who were more premature than in the present series.

Indeed, the thrombin time was the same for mature infants and for those having a birth weight averaging 1780 g.

In all three groups of mothers (at term, about 5 weeks and about 6 weeks before term) the TAT three-stage showed coagulation-activating components of approximately the same magnitude The same applies to the three groups of infants. This supports the theory that it is the mechanism of delivery which is the provoking factor For a more detailed discussion of these findings, *cf Nielsen* (1969 a 1969 b)

The reduced content of coagulation factors II, V VII, and X in the premature infants may be partly explained as a consequence of their immature liver function, and partly because of consumption, induced by coagulation-activating components in the plasma. The same explanation may apply to the reduction of factors VIII and IX whose origin is unknown.

If fibrinogen were synthesized together with the other factors in the li er t would also have been expected to be reduced, but no such reduction was demonstrable.

two-stage with a significantly prolonged recalcification time (0 05 > p > 0 02) while the TAT three-stage curve showed no major changes. PTT was significantly prolonged (0 02 > p > 0.01) and the thrombin time was again at the same level as for normal infants born at term. Otherwise there were no significant changes in coagulation or fibrinolysis.

Discussion

First phase of coagulation. The present investigations confirm previous reports that the platelet count does not alter through the last trimester of pregnancy On the basis of the methods used, which are screening tests it is established that the content of the factors which make up the first phase of coagulation is increased in prematurely delivered women as well as in women who are delivered at term—*cf* the PTT and TAT two-stage, including the recalcification time However it is not possible to decide neither from the literature nor from the present results whether this applies to factor VIII as well as factor IX or merely to one of them.

The platelet count in the cord blood did not differ significantly in the three groups of newborn infants. However there is a tendency to lower values in the premature groups. This is in keeping with the results of *Hansen* (1960) although the absolute figures are not comparable. For factors VIII and IX, there is presumably a question of marked reduction of both most marked in the most premature group.

Second phase of coagulation In conformity with other workers an evenly increasing PP has been demonstrated throughout the last months of pregnancy The negligible differences in the Quick times are presumably due to the unchanged content of factor V throughout pregnancy This too accords with previous reports.

In the newborn the present semiquantitative analytical methods could demonstrate a decreasing content of the factors in the second phase of coagulation (*viz* factors II V VII and X) with increasing prematurity This is further confirmed by the increasing flattening of the TAT two-stage curve which occurs

with increasing prematurity. Accordingly the results correspond with those in Forgács et al.'s (1962) comprehensive study and appear to be in keeping with most of the findings reported in the literature.

Third phase of coagulation. As is apparent from the submitted literature, the fibrinogen content increases as pregnancy advances. In this material, too, there was an increased content in women after delivery but this increase was of the same magnitude in women who were delivered at term as in those who went into labour about 6 weeks before the estimated date of confinement. Thus, the maximum fibrinogen content appears to be attained at a fairly early stage of the third trimester. The thrombin time was also the same in all three groups of delivered women.

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Indeed, the thrombin time was the same for mature infants and for those having a birth weight averaging 1780 g.

In all three groups of mothers (at term, about 5 weeks and about 6 weeks before term) the TAT three-stage showed coagulation-activating components of approximately the same magnitude. The same applies to the three groups of infants. This supports the theory that it is the mechanism of delivery which is the provoking factor. For a more detailed discussion of these findings, cf. Nielsen (1969 a, 1969 b).

The reduced content of coagulation factors II, V, VII and X in the premature infants may be partly explained as a consequence of their immature liver function, and partly because of consumption, induced by coagulation-activating components in the plasma. The same explanation may apply to the reduction of factors VIII and IX whose origin is unknown.

If fibrinogen were synthesized together with the other factors in the liver, it would also have been expected to be reduced, but no such reduction was demonstrable.

Fibrinolysis

The present studies of fibrinolysis showed no significant difference in fibrinolytic activity or in plasminogen content between mothers who went into labour prematurely and mothers who went to term. This agrees with the findings during delivery (*Markanan et al* 1967) The results presented here of fibrinolytic activity in the plasma and in iso-electrically precipitated plasma on standard plates were the same for mothers at term as those reported by *Nilsson* and *Kullander* (1967)

In accordance with a number of previous reports, a reduced fibrinolytic activity was found in the cord blood of premature babies, in unprecipitated plasma as well as in the euglobulin fraction. These observations differ from those of *Forgács et al.* (1962) who found a normal fibrinolytic activity in premature babies. The explanation may be that their series included very small infants, weighing 750 g These infants may have been hypoxic when the blood samples were drawn, and in that event fibrinolytic activity is increased.

The low plasminogen content in the premature babies is in accordance with the results from the literature. The explanation must be poorly developed hepatic function rather than autodigestion caused by spontaneous activation during delivery as *Quie* and *Wannamaker* (1960) have demonstrated that this reduction persists for a least 6 weeks after birth.

A reduced fibrinolytic activity and a low plasminogen content may according to *Ambrus et al.* (1963) involve a danger of hyaline membrane formation. As already mentioned their investigation showed a marked reduction of fibrinolytic activity in premature babies, and particularly low values in infants having hyaline membrane disease. It is assumed that the reduced activity may prevent the dissolution of fibrin which is the basic element of the hyaline membrane On the other hand, *Lieberman* and *Kellogg* (1961) believe that hyaline membrane formation is due to the presence of an activator inhibitor in the lungs.

SUMMARY

In a study of coagulation and fibrinolysis conditions in prematurely delivered women and their premature infants the author made the following findings, compared with normal women delivered at term and their full-time infants

A. *Maternal findings.* A tendency to a prolonged recalcification time and a somewhat lower PP per cent in the prematurely delivered women. In both groups coagulation-activating components were found in the plasma.

Otherwise, no differences in the coagulation or fibrinolysis conditions. No difference in haematocrit.

B *Findings in the newborn.* A highly prolonged recalcification time and a greatly reduced PP per cent plus a highly prolonged PTT in the premature babies. On the other hand, no changes in platelet count or fibrinogen content. The changes were most marked in the most premature infants.

In mature as well as in premature infants the TAT three-stage revealed coagulation-activating components in the plasma.

A distinct reduction in fibrinolytic activity and a reduction in plasminogen level was demonstrated in the premature babies. No difference in the haematocrit was found.

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INFLUENCE OF PRE ECLAMPSIA UPON COAGULATION AND FIBRINOLYSIS IN WOMEN AND THEIR NEWBORN INFANTS IMMEDIATELY AFTER DELIVERY

By

NIELS CHR. NIELSEN

Intravascular haemolysis and changes in coagulation and fibrinolysis have long been known as complications of eclampsia. Alterations in the parameters of coagulation and fibrinolysis have also been reported in pre-eclampsia, but so far only case reports or studies of individual factors have been published. There do not seem to be any studies on the possible influence of pre-eclampsia upon coagulation and fibrinolysis in the newborn apart from a single paper by *Villalba Trininos* (1967) on the fibrinogen content.

Assessment of the available literature causes considerable difficulties, because very often the composition of the series it not clearly defined. Accordingly the results are frequently based upon cases of pre-eclampsia as well as patients with chronic hypertension, chronic pyelonephritis, chronic glomerulonephritis, etc etc.

The object of the present study is to throw some light upon coagulation and fibrinolysis in a well-defined group of mothers with pre-eclampsia and their newborn infants.

Previous Investigations

In the review of the literature the author has included as far as possible only the results of studies on patients who fulfil the same criteria of pre-eclampsia as those in the present series (*cf* "Material and Methods") It was tried also to exclude cases of eclampsia.

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To my knowledge there have been no studies of the platelet count in the newborn infants of pre-eclamptic mothers, and there do not seem to have been any studies made of the other factors involved in the first phase of coagulation either in mothers or infants.

Second Phase of Coagulation

Little has been published concerning the second-phase coagulation factors. *Stefanini* and *Dameshek* (1955) stated that hypoprothrombinaemia was often present in pre-eclampsia, but without further elaborating on the subject. On the other hand, *Szirmai* (1956) on the background of a study by *Arvay* and *Szirmai* (1951) established that in toxaemia the blood level of prothrombin was higher than in normal pregnant women. However no accurate data are given concerning the results or the composition of the series. In respect of factor VII (proconvertin) there seems to be only one report, by *Ciulla* (1955) who stated that the values were higher for pre-eclamptics than for normal pregnant women, but without producing further evidence.

There do not appear to have been any studies of these factors on the newborn infants of pre-eclamptic mothers.

Third Phase of Coagulation

Fibrinogen. The fibrinogen content in the blood of women with pre-eclampsia has been adequately investigated. A number of results are listed in Table I. These results are based upon a number of different techniques which are not mentioned, as it is not the absolute values, but the difference between normal pregnant women and pre-eclamptics which is of interest. The exact composition of the groups of patients is often unknown, as it is not stated by *Vara Kishore et al.* or by *McKay* and *Corey* while *Mack et al. Dieckmann Alvarez* and *Afonso* and *Searle* use, in broad terms, the definition of pre-eclampsia of *The American Committee on Maternal Welfare*

As may be seen from Table I, there is hardly any significant difference in fibrinogen content between normal and pre-eclamptic women, before during or after labour

First Phase of Coagulation

Platelets In a study of 10 patients having moderate toxaemia diagnosed on the basis of moderate hypertension, albuminuria, and varying degrees of oedema" *Ward* and *MacArthur* (1948) demonstrated an average platelet count of 155 000/mml, i.e in the same range as in a control series of normal pregnant women. On the other hand, there was reduction in the platelet count of "six severe pre-eclamptic and one eclamptic this group showed a higher elevation of blood pressure and increase in the amount of albuminuria, and a marked degree of oedema the mean value being stated as 97 000 *Dieckmann* (1952) who used the criteria of pre-eclampsia set up by *The American Committee on Maternal Welfare* with minor modifications reported no difference in platelet counts between normal pregnant women and pregnant women with pre-eclampsia His report is based upon only a few examinations and the exact values are not mentioned. *Pritchard et al.* (1954) found no thrombocytopenia in 13 pre-eclamptic patients the criterion being fixed as a platelet count below 100 000/mml. However in some of their patients they observed a fall from approx. 150 000 to 110 000–120 000 followed by an increase to 200 000 or over after delivery These authors do not state their diagnostic criteria *Ferguson* (1956) studying 16 pre-eclamptics found no major difference in the platelet count as compared with normal pregnant women. Pre-eclampsia, they state, was defined as "persistent hypertension (140/90) or albuminuria that first appeared after the twenty-fourth week of pregnancy and disappeared soon after delivery The same result was reported by *Szinnyai et al* (1962) in a series of 20 pre-eclamptic patients but the basis for the diagnosis is not stated, and by *Zielińska* and *Solecka* (1962) in 21 pregnant patients with slight toxaemia (blood pressure 130/90–170/100) In five patients with severe toxaemia (blood pressure 170/100–200 100) however the latter authors found a significantly reduced platelet count. *Brain et al* (1967) have published a case of haemolysis and thrombocytopenia in a pre-eclamptic (diagnosed in the 24th week) and tabulated a number of previous case reports on severe pre-eclampsia accompanied by thrombocytopenia.

Fibrinogen in cord blood was studied by *Villalba Trivinos* (1967) who found, in 22 newborn infants of mothers with clinical toxaemia only half the mean value measured in 60 children of normal mothers. He gives no explanation of the massive reduction in the infants of toxaemic mothers.

For further elucidation of the third phase of coagulation there have been a few reports on thrombin time. *Pritchard et al.* (1954) found a prolonged thrombin time in 10 out of 13 patients with pre-eclampsia. *Szirmyai et al.* (1962) found no difference in thrombin time between normal and toxaemic pregnant women. *Searle* (1966) comparing 37 patients with mild pre-eclamptic toxaemia with 24 normal pregnant women, concluded that the thrombin time tended to be prolonged in the pre-eclamptic group. This conclusion is based upon the following mean thrombin times control group 9 12 (±0.211) sec. test group 9 46 (±0.904) sec. However as already mentioned, only the last mentioned report was based upon a well-defined group of patients.

To my knowledge there have been no reports on thrombin times in the newborn infants of pre-eclamptic mothers.

Haematocrit It has been pointed out by *Berlin et al.* (1952) and *Dieckmann* (1952) that pregnant women with severe pre-eclampsia have haemoconcentration. In mild pre-eclampsia, on the other hand, there does not seem to be any difference in the haematocrit as compared with normal pregnancy (*Dieckmann* 1952) *Chowdhury* (1961) found no difference in a study of normal and pre-eclamptic mothers (without further substantiating the diagnosis) On the other hand, the haematocrit in the cord blood of the newborn infants of pre-eclamptics was a more recent study *Henger* (1967) comparing 19 pre-eclamptics (defined as at least 2 of the 3 symptoms blood pressure above 140/90 proteinuria exceeding 0.5 g/l, and oedema) and 20 normal pregnant women, found no difference in the haematocrit.

Fibrinolysis. There seems to have been no systematic study of the fibrinolytic system in a well-defined group of patients with pre-eclampsia or their newborn infants. *Smith* and *Smith* (1945) and *Smith* (1947) assumed that in toxaemia hormone production

Table I. *Plasma Fibrinogen Content During Late Pregnancy Delivery and Puerperium (mg/100 ml)*

Authors	Diagnosis	Results $\bar{x}$ and n	Range SD	Time
Mack et al. (1951)	normal pregnancy	610 17	320–840	delivery
	mild pre-eclampsia	640 8	490–890	delivery
	severe pre-eclampsia	670 9	440–1010	delivery
Dieckmann (1952)	normal pregnancy	480	300–700	at term
	pre-eclampsia	510	300–900	at term
Vara (1958)	normal delivery	578 34		immediately post partum
	mild pre-eclampsia	514 23		immediately post partum
Kishore et al (1962)	normal pregnancy	551 53	420–694 ±53	immediately post partum
	pre-eclampsia	599 8	531–683 ±57	immediately post partum
Alvarez and *Afonso* (1964)	normal pregnancy	390 17	±80	1st–6th day post partum
	mild pre-eclampsia	410 20	±120	1st–6th day post partum
	severe pre-eclampsia	442 19	±150	1st–6th day post partum
McKay and *Corey* (1964)	normal pregnancy	522 6	412–655	1st day post partum
	pre-eclampsia	547 6	434–680	post partum
Searle (1966)	normal pregnancy	400 24	±47	late pregnancy
	mild pre-eclampsia	500 37	±80	late pregnancy

$\bar{x}$ = arithmetic average n = number of estimations
range = range of individual results SD = standard deviation

Table III. Results of Coagulation and Fibrinolysis Studies in Newborn Infants

Tests see Methods	Normal Infants		Infants of Pre-Eclamptic Mothers		Premature Infants	
	x̄ and n	Range est. SD	x̄ and n	Range est. SD.	x̄ and n	Range est. SD
Platelets (thousands	323	86–571	217	108–321	307	174–625
per cu mm.)	n=20	±147	n=10	±68	n=10	±144
Proaccelerin	115	62 170	91	70–120	92	59–115
(per cent)	n=20	±34	n=10	±17	n=10	±21
Prothrombin time	19	17–22	21	19–25	19	17 23
(seconds)	n=20	±1.5	n=10	±1.8	n=10	±2.1
P and P	72	30–115	46	34–79		
(per cent)	n=20	±25	n=10	±13		
P and P*	90	62 150			44	27–78
(per cent)	n=20	±21			n=10	±16
Partial thromboplastin	88	78–125	106	83–128	112	97 144
time (seconds)	n=20	±11	n=10	±18	n=10	±15
Thrombin time	13	10–15	19	11 31	10	7 14
(seconds)	n=20	±1.5	n=10	±7.0	n=10	±2.3
Fibrinogen	242	150–340	177	85–250	264	140–370
(mg per 100 ml)	n=20	±50	n=10	±59	n=10	±67
Haematocrit	55	48–63	57	50–62	56	47–65
(per cent)	n=20	±4.9	n=10	±3.7	n=10	±5.6
Standard fibrin plates	86	16–230	49	0–121	38	0–54
Untreated plasma						
(sq mm)	n=20	±56	n=10	±44	n=10	±22
Standard fibrin plates	390	251–658	251	0–589	211	25–324
Euglobulins (sq mm)	n=20	±119	n=10	±192	n=10	±104
Heated fibrin plates	25	9–49	19	0–36	12	0–25
Untreated plasma						
(sq mm)	n=20	±9.7	n=10	±14	n=10	±8.3
Heated fibrin plates	34	19–49	24	0–40	27	1–49
Euglobulins (sq mm)	n=20	±7.8	n=10	±13	n=10	±12
Plasminogen (µg Co-	67	37 108	55	19–102	33	17–46
[illegible] per ml)	n=20	19	n=10	±28	n=10	±12

See footnote to Table II

Prophylactic treatment of the mothers with vitamin K

Table II. *Results of Coagulation and Fibrinolysis Studies in Normal and Pre-Eclamptic Women Immediately after Delivery*

Tests see Methods	Normal Women at Term		Pre-Eclamptic Women 2–3 Weeks Before Term		Normal Women 5 Weeks Before Term	
	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD	$\bar{x}$ and n	Range est. SD
Platelets (thousands	207	67–351	152	113–188	231	139–448
per cu. mm.)	n = 20	±76	n = 10	±26	n = 10	±90
Proaccelerin	110	80–190	118	102–145	101	15–125
(per cent)	n = 20	±31	n = 10	±15	n = 10	±17
Prothrombin time	17	16–20	17	16–19	18	15–20
(seconds)	n = 20	±0.9	n = 10	±0.9	n = 10	±1.7
P and P	206	120–380	190	162–225	1 8	115–245
(per cent)	n = 20	±68	n = 10	±17	n = 10	±44
Partial thromboplastin	73	66–79	77	71–83	75	67–55
time (seconds)	n = 20	±3.6	n = 10	±3.6	n = 10	±5.0
Thrombin time	8	5–10	7	5–8	6	5–8
(seconds)	n = 20	±1 1	n = 10	±1 1	n = 10	±1 0
Fibrinogen	508	320–965	466	340–595	515	440–650
(mg per 100 ml)	n = 20	±154	n = 10	±82	n = 10	±57
Haematocrit	42	36–48	41	34–47	41	35–55
(per cent)	n = 20	±3.2	n = 10	±4 7	n = 10	±1.8
Standard fibrin plates	0	0	0	0	0	0
Untreated plasma						
(sq mm.)	n = 20	±0	n = 10	±0	n = 10	±0
Standard fibrin plates	39	0–125	28	0–49	30	0–93
Euglobulins (sq mm.)	n = 20	±25	n = 10	±15	n = 10	±35
Heated fibrin plates	0	0	0	0	0	0
Untreated plasma						
(sq mm.)	n = 20	±0	n = 10	±0	n = 10	±0
Heated fibrin plates	27	0–49	26	4–45	21	0–40
Euglobulins (sq mm.)	n = 20	±14	n = 10	±14	n = 10	±14
Plasminogen (µg Cu-	173	108–234	161	130–214	169	126–198
Tyrosine per ml.)	n = 20	±25	n = 10	±26	n = 10	±18

$\bar{x}$ = arithmetic average n = number of estimations
range = range of individual results (x) est. SD = estimated tandard deviation
$= \pm \sqrt{\frac{\Sigma (x-\bar{x})^2}{n-1}}$

One patient went into labour spontaneously and had a normal delivery 3 had a normal delivery after the membranes had been ruptured, 3 had forceps deliveries, and 3 had Caesarean sections (2 after the onset of labour and 1 elective) In 5 cases prophylactic vitamin K was administered prior to delivery in doses as described previously (*Nielsen* 1969 a) Delivery occurred on average 2 or 3 weeks before term (range 0–6 weeks) The blood samples were drawn within the first half-hour after delivery Moreover blood samples were removed from these patients newborn infants within the first 20 minutes after birth. Birth weight averaged 2690 g (range 1500–4150 g) During the stay in the ward the infants showed no abnormalities, apart from the signs of prematurity

Group 2 The control series comprised, firstly a group of 20 normal delivered women at term and their normal newborn infants these women did not receive prophylactic vitamin K. Also, a group of 10 women treated prophylactically with vitamin K, delivered about 5 weeks (range 4–7 weeks) before calculated term, but having an otherwise normal pregnancy deli ery and puerperium, and their premature infants (mean birth weight 2270 g (range 2070–2400 g)) These control series have been described previously (*Nielsen* 1969 a 1969 b)

The maternal blood samples were drawn from an ante-cubital vein after the application of light tourniquet. In the newborn the umbilical cord was incised close to the skin, and disposable plastic catheter was inserted through the umbilical vein The samples were used only if there was free flow of blood through the catheter The technique of the blood sampling and the further treatment of the samples have been described previously Determination of the recalcification time, the thromboplastin activation test (TAT) two- and three-stage, partial thromboplastin time (PTT) thrombin time prothrombin time (Quick) factor V prothrombin-proconvertin (PP) fibrinogen content, and measurement of fibrinolytic activity in plasma and in iso-electrically precipitated plasma on untreated fibrin plates (standard plates) and on heated fibrin plates as well as plasminogen, haematocrit, and platelet counts were also performed as described previously (Nielsen 1969)

Results

The results of the coagulation studies are given in Tables II and III those of the TAT and recalcification times in Figs 1 2, 3 4 5

was reduced and that this resulted in increased catabolism and the formation of a "toxin" supposed to activate coagulation and increase fibrinolytic activity In accordance with this theory they found an increased fibrinolytic activity in euglobulin serum from 11 pre-eclamptic or eclamptic patients in the second and third trimester of pregnancy on comparison with 6 normal pregnant women. However these results must be considered with reserve as the patients were in the second or third trimester i.e some of them at a stage of pregnancy when the diagnosis of pre-eclampsia cannot normally be accepted Moreover the series includes an unknown number of eclamptic patients.

Willson and *Munnel* (1946) found no fibrinolytic activity in the peripheral blood of normal pregnant women while fibrinolysis occurred in chronic vascular-renal diseases associated with proteinuria and oedema as well as in pre-eclampsia. More recent studies by *S innayi et al.* (1962) and *Searle* (1966) have shown no difference in fibrinolytic activity between normal and pre-eclamptic pregnant women, but their reports too are based upon very rough screening of the fibrinolytic system.

Material and Methods

The analyses were performed on blood samples from the following two groups

Group 1 Ten women (9 primiparae and 1 para-2) with pre-eclampsia selected according to the following criteria
(a) healthy before the pregnancy (b) normal blood pressure i.e in no case exceeding 130 mm Hg systolic or 90 mm Hg diastolic during the first two trimesters of pregnancy (c) increasing blood pressure during the third trimester calling in all cases for treatment with pethidine-chlorpromazine or Stroganoff (mean blood pressure on medication at time of delivery 180/110 mm Hg (range 160/90–205/120 mm Hg) (d) simultaneously with the increase in blood pressure oedema and proteinuria (at the time of delivery a mean of 0 5 per cent (range 0 04–1 4 per cent)] (e) blood pressure returned to normal and proteinuria subsided after delivery so that at follow-up about 8 weeks after delivery the patient had normal blood pressure and no proteinuria.

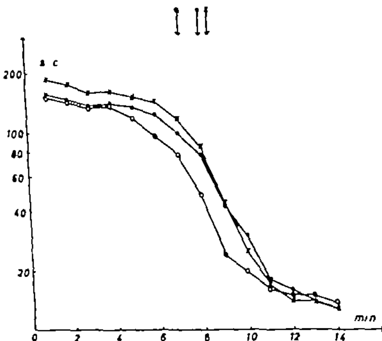


Fig. 2. Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women with pre-eclampsia.
—— 10 normal adult women, two-stage TAT
—— 10 women with pre-eclampsia, two-stage TAT immediately after delivery
—— 10 women with pre-eclampsia, three-stage TAT immediately after delivery

no major differences between the two groups. However t max was shortened in pre-eclamptic mothers ($0.05 > p > 0.02$). By TAT three-stage, coagulation-activating components in the same range were found in the plasma from both groups.

B. *Cord blod.* In the newborn infants of pre-eclamptic mothers the coagulation factors were considerably reduced. This was manifested by a prolonged Quick time ($0.01 > p > 0.001$), a reduced PP level ($p < 0.001$), factor V level ($0.05 > p > 0.02$), fibrinogen ($0.01 > p > 0.001$) and platelet count ($0.02 > p > 0.01$) simultaneoulsy with a prolonged PTT ($0.01 > p > 0.001$) and

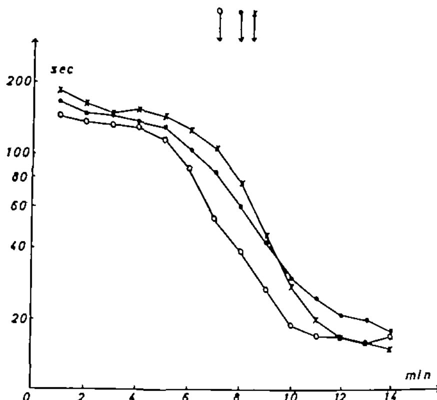


Fig. 1 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 20 normal women at term.
x ——— x 15 normal adult woman, two-stage TAT
• ——— • 20 normal women two-stage TAT immediately after delivery
o ——— o 20 normal women, three-stage TAT immediately after delivery

and 6 Since the PP level in the newborns is the only parameter of coagulation fibrinolysis which is susceptible to the administration of vitamin K to the mothers (*Nielsen* 1969 a) these values, for the newborn of untreated as well as treated mothers, are given in Table III.

Comparison of normal mothers at term and their newborn infants with pre-eclamptic mothers and their newborn infants revealed the following significant differences

A. *Peripheral maternal blood* A reduced platelet count in the pre-eclamptic mothers ($0\,01 > p > 0\,001$) and a prolonged PTT ($0\,01 > p > 0\,001$) In addition, a shortened thrombin time in the pre-eclamptics ($0\,01 > p > 0\,001$) TAT two-stage showed

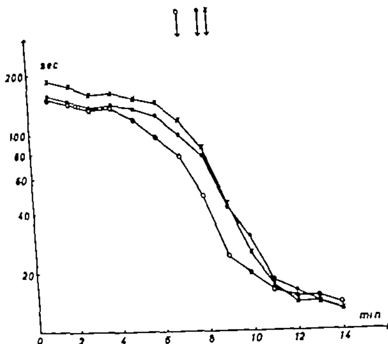


Fig 2 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women with pre-eclampsia.
—x 10 normal adult women, two-stage TAT
•— 10 women with pre-eclampsia, two-stage TAT immediately after delivery
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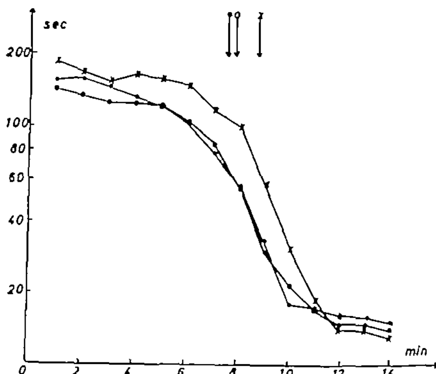


Fig 3 Thromboplastin activation curve of peripheral blood drawn immediately after delivery from 10 women an average of five weeks before estimated date of confinement.

x ——— x 10 normal adult women, two-stage TAT
• ——— • 10 women two-stage TAT immediately after delivery
o ——— o 10 women, three-stage TAT immediately after delivery

thrombin time (0 01 > p > 0 001) In accordance with these findings the TAT two-stage curve was flatter than the corresponding curve for normal infants. At the same time the recalcification time was prolonged but not significantly from 565 sec. to 620 By the TAT three-stage coagulation activating components were demonstrated in the plasma from both groups, possibly more marked in the newborn infants of pre-eclamptic mothers.

The results of the *fibrinolysis studies* are also given in Tables II and III. A comparison of the same groups showed

A *Peripheral maternal blood* No significant difference

B *Cord blood* Significantly reduced fibrinolytic activity in the plasma euglobulin fraction in the newborn infants of pre-

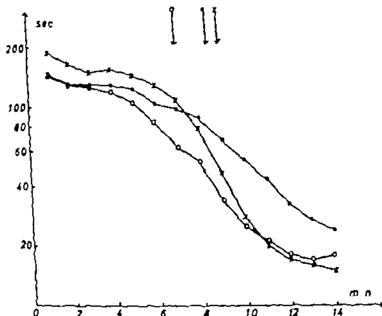


Fig. 4 Thromboplastin activation curve of venous cord blood from 20 normal newborn infants

—— x 15 normal adult women, two-stage TAT
—— • 20 normal infants, two-stage TAT
—— 20 normal infants, three-stage TAT

eclamptic mothers upon determinations on standard fibrin plates ($0.05 > p > 0.02$) as well as on heated fibrin plates ($0.05 > p > 0.02$)

Since the duration of pregnancy influences the factors concerned with coagulation and fibrinolysis, Tables II and III also give the results for a group of mothers delivered approximately 5 weeks before term and their premature infants, selected as described under "Material and Methods

Comparison of the group of pre-eclamptic mothers, who were delivered 2 or 3 weeks before term, with a group of mothers who were delivered approximately 5 weeks before term showed that the above-mentioned differences in platelet count cannot be explained as a result of the duration of pregnancy since the platelet counts in the pre-eclamptics are also significantly lower than

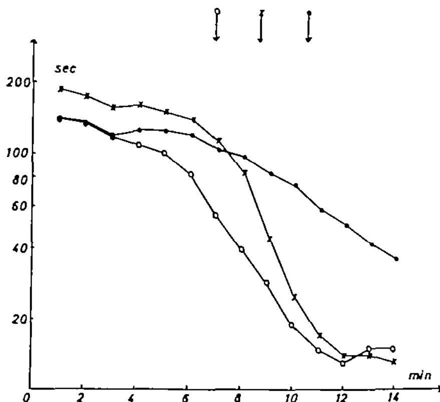


Fig. 5. Thromboplastin activation curve of venous cord blood from 10 newborn infants of pre-eclamptic mothers.
x ——— x 10 normal adult women, two-stage TAT
● ——— ● 10 infants of pre-eclamptic mothers two-stage TAT
o ——— o 10 infants of pre-eclamptic mothers three-stage TAT

those of the prematurely delivered, otherwise normal women (0 05 > p > 0 02) On the other hand, this comparison showed no significant differences in PTT or t max and the thrombin time of the pre-eclamptics was midway between the values for women delivered at term and prematurely TAT two- or three-stage showed no major differences between pre-eclamptics and prematurely delivered women. However according to the TAT three-stage there seems to be a tendency to a more pronounced activation in the pre-eclamptics.

Newborn infants of pre-eclamptic mothers have a prolonged thrombin time (0 01 > p > 0 001) and a reduced fibrinogen content (0 01 > p > 0 001) whereas other factors are in the same

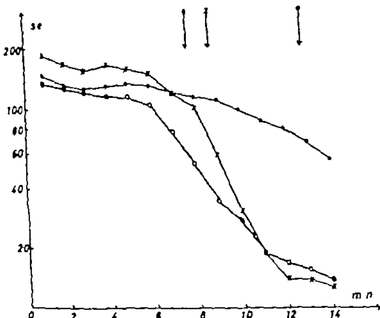


Fig 6 Thromboplastin activation curv of venous cord blood from 10 premature newborn infants

x —— 10 normal adult women, two-stage TAT
—— 10 premature infants two-stage TAT
• —— • 10 premature infants, three-stage TAT

range as in premature infants of normal mothers. The TAT two-stage curve was not quite as flat in the infants of pre-eclamptic mothers as in the otherwise normal premature infants and the recalcification time was shorter but not significantly 620 sec. as compared with 770 sec. According to the TAT three-stage there seems to be a somewhat more pronounced coagulation activity in the plasma of the newborn infants of pre-eclamptic mothers than in the premature infants.

Fibrinolytic activity in the newborn infants of pre-eclamptic mothers and in premature infants proved to be in the same range only the content of plasminogen was significantly reduced in the premature group ($0.05 > p > 0.02$)

Discussion

The present results do not indicate any major difference in the factors concerned with coagulation and fibrinolysis between normal mothers and pre-eclamptic mothers. This is confirmed by a study (*Skjodt* personal communication) on the parameters of coagulation and fibrinolysis in pre-eclamptics before, during, and after delivery

The relatively low platelet count in the pre-eclamptics is possibly explained by consumption due to a slightly increased activation of the coagulation system. This idea is supported by the report of *McKay et al.* (1964) who demonstrated increased platelet adhesiveness in pre-eclamptic patients, interpreted as a sign of a "lower grade process of disseminated intravascular coagulation in pre-eclampsia

The reported results concerning platelet counts in pre-eclamptics are not consistent perhaps because of the heterogeneous groups studied. However the platelet count seems to be reduced in severe forms of pre-eclampsia a tendency confirmed by the present investigations.

The presence of mild intravascular coagulation may also explain the prolonged PTT and the slightly reduced fibrinogen level in pre-eclamptics.

Other parameters of coagulation and fibrinolysis proved to lie, as expected, between those for mothers delivered at term and mothers delivered approx. 5 weeks before term. A prolonged thrombin time or changes in fibrinolysis could not be demonstrated.

The infants of pre-eclamptic mothers on the other hand, show considerable changes in the parameters of coagulation. These changes may be interpreted as the consequences of a degree of intravascular coagulation, as manifest by a reduction in the platelet count of the PP factor V and fibrinogen levels, as well as in a prolongation of PTT Quick time and thrombin time The course of the TAT three-stage curve is also compatible with an increased content of coagulation activating components in the plasma.

The reduced content of coagulation factors in the infants of

pre-eclamptic mothers cannot be explained by birth at an average of 2 or 3 weeks before term, as the values were in several cases even lower than in infants born, on average 5 weeks *prematurely* to normal mothers.

Fibrinolysis does not seem to undergo major changes apart from those which may be ascribed to slight prematurity

A number of other publications have supported the assumption of slight intravascular coagulation in pre-eclamptic mothers. This applies to *McKay* and *Corey* (1964) on the background of their study of cryofibrinogen, and to *Mautner et al.* (1962) *Beller* (1964) and others who have demonstrated subendothelial depositions of fibrin in the capillary coils of the renal glomeruli.

As pointed out by *McKay et al.* (1964) it must be emphasized that intravascular coagulation in eclampsia is different from that in pre-eclampsia. In eclampsia there is a sudden and massive process leading to capillary thrombosis, visible in the light microscope. In pre-eclampsia, on the other hand coagulation is slower and incomplete, so that capillary occlusion does not occur but, as already mentioned, there is a deposition of fibrin on the basement membrane of the renal glomeruli.

The cause of this intravascular coagulation is uncertain. *Schneider* (1951 1952) suggested liberation of thromboplastin from a retroplacental or intradecidual haematoma or tissue thromboplastin of placental origin. As stated by *McKay et al.* (1964) the cause may also be coagulation-activating components released from platelets which have been destroyed by adhering to the syncytial trophoblast which is damaged for some unknown reason.

If one of these theories is correct, there ought to be a possibility of intravascular coagulation also in the newborn infant. Indeed, *Leissring* and *Vorlicky* (1968) have recently published a case in which coagulation studies and later autopsy of the newborn infant of a pre-eclamptic mother showed massive intravascular coagulation

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The reduced content of coagulation factors in the infants of

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livered at term and their normal infants showed

A. *In the mothers.* Reduced platelet count prolonged PTT and a slighly reduced fibrinogen content. These results are compatible with mild intravascular coagulation.

Otherwise no differences in the parameters of coagulation or fibrinolysis and no difference in haematocrit could be shown.

B. *In the newborns.* In the infants of pre-eclamptic mothers a considerable reduction in platelet count, PP factor V and fibrinogen content as well as prolonged PTT Quick and thrombin time The TAT three-stage showed a slightly increased content of coagulation-activating components in the plasma. These results might indicate that some degree of intravascular coagulation had taken place.

There were no changes in fibrinolytic activity apart from what may be due to the slight prematurity There was no difference in the haematocrit.

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INCORPORATION OF TETRACYCLINE INTO HUMAN FOETAL BONES AFTER MATERNAL DRUG ADMINISTRATION

BY

L. ERIK TÖTTERMAN[1] AND LAURI SAXÉN

It has long been known that tetracyclines are incorporated into growing bones (*Milch et al* 1957 *Frost et al* 1960) and that at high dosages they are deleterious to bone development in chick embryos (*Bevelander et al* 1960) Clinically acceptable doses do not seem to have any morphologically demonstrable persistent effect on bone growth (*Hughes et al.* 1965) *Charles* (1954) demonstrated placental transmission of tetracyclines and *Gibbons* and *Reichelderfer* (1960) found that the concentration of tetracycline in cord blood sometimes exceeded the values noted in premature infants given peroral tetracycline medication. Therefore it might be expected that the administration of tetracycline during mammalian pregnancy would result in demonstrable effects on the foetal bones. *Cohlan et al* (1963) showed that rat embryos like chick embryos were affected by early protracted and heavy administration of tetracycline during pregnancy These investigators also detected tetracycline (as judged by fluorescence) in all investigated bones of a premature human foetus whose mother had been given tetracycline prior to delivery and *Douglas* (1963) observed fluorescence in the deciduous teeth of infants whose mothers had received tetracycline during pregnancy However in a controlled study of the fluore-

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Fig 1 A section of the distal phalangeal bone of the digit of full-term infant of mother who received tetracycline just prior to delivery (Case B Table II) Photographed in UV-light Yellow fluorescence is seen at the newly mineralized zones of the bone

scence in the deciduous teeth after prenatal, long-term administration of tetracycline, no effect was observed when the medication had been given during the first trimester of pregnancy and fluorescence was not invariably noted with certainty even after protracted tetracycline administration during the last trimester (Porter et al. 1966) Thus considerable indi idual ariations seem to occur in the relationship between the amount of tetracycline administered and the amount of fluorescence observed subsequently in the foetus or infant. For this reason it was thought useful to study a series of early foetuses, and some still

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Fig 1 A section of the distal phalangeal bone of the digit of full-term infant of mother who received tetracycline just prior to delivery (Case B Table II) Photographed in UV light Yellow fluorescence is seen at the newly mineralized zones of the bone.

scence in the deciduous teeth after prenatal, long-term administration of tetracycline no effect was observed when the medication had been given during the first trimester of pregnancy and fluorescence was not invariably noted with certainty even after protracted tetracycline administration during the last trimester (*Porter et al.* 1966) Thus considerable individual variations seem to occur in the relationship between the amount of tetracycline administered and the amount of fluorescence observed subsequently in the foetus or infant. For this reason it was thought useful to study a series of early foetuses, and some still-

births and neonatal deaths whose mothers had received tetracycline medication during pregnancy

Material and methods

During the years 1962–1966 foetuses were collected after therapeutic abortions. The main indication for termination of pregnancy was protracted physical or mental strain, but as a rule there was also some medical indication of minor importance. In 23 cases it was known that the mother had received tetracycline during pregnancy In addition there were one full term and one premature infant who died postnatally and one premature foetus who died *in utero*

The small foetuses with a menstrual age from 82 to 128 days (Table I) obtained by hysterotomy were stored in 10 % formalin for later investigation. Longitudinal sections of the tibia were taken for the study of fluorescence. In the older foetus and the two infants (Table II) bone tissue from the fifth toe was examined Undecalcified samples were sectioned in paraffin and examined unstained in UV light under a Leitz Panphot microscope.

The mothers had been given one of the following tetracycline compounds tetracycline (TC) oxytetracycline (OTC) rolitetracycline (RTC) In addition tetracycline (TC) coupled to a side-chain L-methylenelysine (*de Carneri* 1961) had been administered in four cases (nos. 170 156 159 and 155) As appears from Tables I and II the series is not homogeneous in respect to dosage or duration or mode of administration but the time of the therapeutic termination of pregnancy at a menstrual age of about 120 days, was more or less the same in all cases except one (no 156)

Results

The microscopic observations on the presence or absence of fluorescence in the foetuses and infants examined are indicated in Tables I and II where other details of the tetracycline treatment are also given.

Table I. The Young Foetuses Listed According to the Menstrual Age of the Last Known Period of Tetracycline Medication. Each Point in Column 3 Stands for One Day of Tetracycline and the Vertical Lines Indicate the Time of the Therapeutic Abortion. The Findings i.e. the Presence of Fluorescence Appear in Column 4

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
149	m		DISTINCT	TC 0.25×4 b TC 0.5	6	H C
1	m			TC 0.5×4	4	C
185				OTC 0.1 2 m	0.4	C
143	m			TC 0.5×4	4	C
63	m		THIN	TC 0.25×4	3	C
168	m		VERY CLEAR	TC 0.25×4 b TC 0.5 4	6	H C
54	m			OTC 0.25×4	6	H
164				TC 0.25× b TC 0.5	8	H C
63	m			TC 0.25×4	4	
198	m			TC 0.5 3	3	C
34				TC 0.25 4		C
180	m			TC 0.25×4	6	H
6	m			TC 0.25×4	4	H
	m		DIFFUSE	RTC 0.3 2	1.2	H
145	m			OTC 0.25×4	2	C
52	m			RTC 0.3 2	1.2	
135	m			TC 0.1 2 m	0	C
59				TC 0.15	3.6	C
58	m			TC 0.1 2 m	0.8	C
50				TC 0.25×4		
206				TC 0.15×4	2	
70				RTC 0.3 2	3.6	
208	m			OTC 0.25×4	8	
		60 80 100 120				

births and neonatal deaths whose mothers had received tetra cycline medication during pregnancy

Material and methods

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149	m		DISTINCT	TC 0.25×4 b TC 0.5 4	6	H C
	m			TC 0.5×4	4	C
165	f		↔	OTC 0.1 2 m	0.4	C
1 3	m			TC 0.5×4	4	C
160	m		THIN	TC 0.25×4	3	C
168	m		↔ VERY CLEAR	TC 0.25×4 b TC 0.5	6	C
154	m			OTC 0.25×4	6	H
64	f			a TC 0.25×4 b TC 0.5 4	8	H C
63	m			TC 0.25×4	4	H
196	m			TC 0.5 3	3	C
34				TC 0.25	4	C
169	m			TC 0.25×4	5	H
148	m			TC 0.25	4	
147	m		DIFFUSE	RTC 0.3 2	2	H
145	m			OTC 0.25×	2	C
42	m			RTC 0.3 2	2	H
155	m			TC 0.1 2 m	0.4	C
159				TC 0.15	3.6	C
56	m			TC 0 2 m	0.6	C
60				TC 0.25 4		
205				TC 0.15×	1	
70				RTC 0.3 2	3.6	
204	m			OTC 0.25×4	6	
		60 80 100 120				

TC-L-methylenelysine

Table II Stillbirths and Neonatal Deaths Menstrual Age of Tetracycline Administration (2) and of Foetal/Neonatal Death (3) Compound of Tetracycline Used (4) Amount Given per Day (5) and Total Amount (6) Fluorescence Reco ded (7) and Some Clinical Comments (8)

Ca e	M n trual Age at Med cation	Menstrual Age at Death	Compound of TC used	Amount per Day	Total Amount (g)	Fluore scence Recorded	Clinical Comments
1	2	3	4	5	6	7	8
A	283–284	287	TC	0.25 × 4	2.5		2300 g. Placental insufficiency(?)
B	282–283	287	TC	0.5 × 6	6.0		27.0 g Anencephalus
C	185–188	215?	RTC	0.3 × 2	2.4	( )	1120 g. Infarcted placenta

Discussion

Among the 23 young foetuses there were only 10 which showed fluorescence. In the group in which no fluorescence was observed the treatment had been given in the patient's home in some cases and in hospital in some. Hence it does not seem probable that the high proportion of cases showing absence of fluorescence is due to carelessness when the medication was given in the patient's home. It may be assumed that there are individual variations in the resorption of tetracycline. The concentration of the drug in the maternal serum varies, and different ratios between the maternal and foetal serum concentrations even seem to occur (*Gibbons* and *Reichelderfer* 1960). This assumption is corroborated by the above-mentioned report by *Porter et al.* (1966) according to which fluorescence in the deciduous teeth was not invariably present when protracted tetracycline treatment had been given during the last trimester. It should also be pointed out that although the interval between the medication and the death of the foetus in the present series was usually short, a few weeks at the most, it nonetheless constituted a considerable proportion of the total life of the foetus, and during this period the affected parts of the skeleton may have undergone considerable resorption. Moreover, in some cases the treatment was possibly commenced well before the onset of bone mineralization, and hence uptake of the drug might not have taken place. After protracted tetracycline treatment during gestation *Johnson* and *Mitchell* (1966) found that the fluorophore disappeared from young rats within three weeks. Considering this and the early time of administration of the tetracycline it is almost surprising that fluorescence was demonstrable in case 170 of the present series. It may be suspected that in this case the tetracycline was ingested later than indicated, or that the patient had received a second course of treatment regarding which no data were available. In the remaining cases showing fluorescence the medication was initiated later and the interval between the termination of medication and the death of the foetus was less than four weeks, *i.e.* at most about half the time indicated in case 170.

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It has been shown that different tetracycline compounds differ in respect to affinity for and effect on the bone tissue (*Tubaro* 1964 *Johnson* and *Mitchell*, 1966 *Saxén* 1966) In this limited series three different compounds of tetracyclines were used, but the number of commercial brands was much higher i.e. eight. Therefore, and considering the lack of homogeneity in regard to dosage and duration and mode of administration, it is impossible to decide whether any particular compound was more likely than the remainder to leave fluorescence in the human foetal bone tissue. On the other hand, it was apparent that even medication of short duration may leave traces (nos. 147 165 141)

SUMMARY

A series of 23 foetuses obtained in therapeutic abortion around the menstrual age of 120 days and three perinatally dead infants were examined for tetracycline-type fluorescence in their skeleton. The mothers had received different tetracycline compounds during pregnancy Fluorescence detected in 10 of the cases confirmed earlier findings on the transplacental transmission of the drug, and indicated that this can take place also during the first trimester Individual variations in the time of administration and in the total dose were considerable but there seemed to be a correlation between the dose and the appearence of bone fluorescence Even short term tetracycline administration resulted in fluorescence in some foetuses No differences between the three different tetracyclines used as regards the incorporation in bone could be shown

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From the Sweden-Ceylon Family Planning Project (Director Carl Erik Groth M.D.)

HISTORY OF CONTRACEPTION AND INTRODUCTION OF INTRA UTERINE CONTRACEPTIVE DEVICES IN A RURAL AREA—BANDARAGAMA CEYLON

BY

L. G. ARUMUGAM

Sir Joseph Hutchinson once remarked 'The great remaining challenge of our time is for Man to master the threat of his own numbers' The modern era of rapid population growth unique both in terms of speed and magnitude in the history of mankind, has its source in the rapid response of the death rate to the modern developments in Science and Technology while the birth rate remains more or less constant. Till quite recently in developing and under-developed countries, survival rather than overgrowth has been the problem In Ceylon the expectation of life which was about 30 years in 1920 has more than doubled itself due no doubt to the spectacular effectiveness with which modern preventive and curative medicine control disease

Population A few points regarding the vital statistics in Ceylon may be relevant. Unlike in many other developing countries there is available in Ceylon a century of records relating to vital events

The enactment of the Births and Deaths Ordinance of 1867 ensured that the occurrence of a birth or deaths is recorded—a birth within 42 days and a death within 5 days as far as the civil population is concerned. There has been also a decennial census examination commencing in 1871

The Table appended hereunder shows the rate of population growth from 1871–1967

Year	Population	Average Growth Rate
1871	2,400,000	1.42
1901	3,658,954	1.72
1931	5,306,871	1.67
1946	6,657,339	1.52
1963	10,644,809	2.75
1967	11,648,148	2.8

After 1946 there is a phenomenal leap upwards and at the present rate of increase, the population will nearly double itself in 25 years. It has even been termed a Demographic Curse for within one year the Island's death rate dropped from 20 per thousand in 1946 to 14.3 in 1947 due in large measure to the dramatic eradication of malaria, the No. 1 killer of that time. Today it is at 8 per thousand, the lowest ever recorded, so that the second half of the present century can be regarded as the epoch of population explosion in Ceylon.

The infant mortality rate declined from 196 per thousand live births in 1900 to 52 in 1967

The maternal mortality rate of 19 per thousand in 1900 has now fallen to 2.8 in 1964 and is at that level still.

A reference to the population pyramid shows some interesting features. There is a high proportion of young children under 15 years constituting 40 per cent of the total population. The productive group—15–60 years—is about 50 per cent, making the dependency rate high as compared with what obtains in the developed countries.

The number in the infant and pre-school group has more than doubled itself since 1900 so that more and more survive to adolescence and old age

The birth rate is about 34 per thousand today A study of fertility trends in Ceylon in 1963–64 revealed that the mean age at marriage was 23.1 in 1960 as compared to 21 in 1900. The total fertility rate was 5.13 in 1952 and 5.07 in 1960. The highest age specific fertility rate was among the group aged 25–29 years.

A sudden increase in the rate of growth of the population necessarily entails serious repercussions, politically economically

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socially and culturally Ceylon is not as yet self-sufficient in rice—the staple food—and imports are necessary, though in the past few years, food production has been stepped up considerably Additional factors involved are increasing unemployment, short age of housing and educational problems which the Government has to contend with.

Since the chief factor in the rapid increase of Ceylon s population is fertility, any measures taken to check this would naturally concern the birth rate and family size There is now a growing awareness of the people to limit the size of their families—this awareness coupled with the governmental effort to disseminate knowledge on planned parenthood, followed by supplies of contraceptives made easily available to the people would produce the desired results.

History of Family Planning in Ceylon The Family Planning Association of Ceylon—a voluntary organization—were the pioneers in this work in Ceylon. Their activities commenced in 1953 and the Association has just celebrated its 15th anniversary In addition to the big, well-attended clinics in Colombo, they also extended their work into other areas established several clinics and took part in the Training Programme for Doctors.

Sweden-Ceylon Family Planning Project This Project commenced its activities in Ceylon after having signed a Bilateral Agreement between the Royal Government of Sweden and the Government of Ceylon in 1958 The terms of the Agreement were for both Governments to co-operate in order to promote and facilitate a pilot project in Community Family Planning in two or more rural areas with a view to extending these activities on a nation wide scale—which has been done since

Of the areas selected one is a village Bandaragama—about 20 miles from Colombo the capital city It is a typical agricultural area with a Sinhalese Buddhist population of just over 7000 the literary rate here being 87 per cent in males and 72 per cent in females.

The second area chosen for its programme of work was the Hindu Indian Tamil populated Tea Estate area of Diyagama, about 100 miles from Colombo The literacy rate here is much lower

After a preliminary census and attitude survey a *Family Welfare Centre* was set up in Bandaragama. The total population in the area at the commencement of activities was 7588 but the Project was concerned only with the Productive Family Group i.e. all couples between the ages of 15 and 40 living as husband and wife and women over 40 who had their last baby within two years and wanted family planning advice. This group is generally 10–12 per cent of the population. The attitude survey revealed that out of 679 families in the Productive Family Group in the village area, 440 or 64.8 per cent were positive to family planning. In 1963–64 75.6 per cent were positive. Families who received more home-visits by the Social Workers remained positive for longer periods.

In regard to the socio-economic background, no difference in attitudes to family planning or fertility was noted.

Education for the lay population was done in the following manner

Wives. The routine ante-natal talks included facts about family planning, physiology of reproduction and how to avoid pregnancy

Midwives continued this education to mothers in the homes during their ante-natal and post-partum visits.

Social workers paid regular visits to the home for purposes of motivation.

Husbands. Every pregnant woman who attended the ante-natal clinic was given some literature on family planning—enclosed in an envelope and addressed to the husband—to be handed over to him.

Group talks were arranged at suitable intervals.

The co-operation of groups of influential men in the village was sought and they helped with the education of the public.

Films were also shown in order to create an interest in family planning

Census An annual census was carried out at the end of each year In order to increase the reliability of the census figures, the project appointed one of its staff members as Census Officer who paid monthly visits to the Registrar of births of the area The Registrar of marriages was also contacted in order to

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Director of Health Services regarding the working and progress of the National Programme.

About two-thirds of the staff have already been trained and 332 clinics have been set up in various parts of the country

If the programme works to schedule, the target can easily be achieved.

The Introduction of the Intra-Uterine Contraceptive in Bandaragama. It was in April 1964 that the I.U.D.-service was introduced in the village area.

Contraception by means of intra-uterine devices is certainly no modern method. For centuries Arabian and Turkish camel-owners have used I.U.D.s to prevent pregnancies in their saddle animals. The technique was simple a small pebble was inserted into the uterus through a hollow tube.

As early as at the beginning of this century German gynaecologists used this method and in 1924 *Grafenberg* reported his results with his silver or silk-worm gut rings.

For various reasons, this method became discredited and was not adopted till about 7–8 years ago and since that time no other contraceptive method has undergone so rapid and thorough a change of medical reputation as that experienced by the I.U.D.s. It is unfortunate that Grafenberg never lived to see the acknowledgement of the value of his idea. There is a saying in Medicine that an idea is never accepted because it is good or logical but because the opposition dies off' Two main factors antibiotics and invention of inert plastic materials are mainly responsible for the revival of interest in the Grafenberg ring and its modifications

In 1959 *Oppenheimer* of Israel and *Ishihama* of Japan published reports on I.U.D.s both authors stressed the low pregnancy rates achieved by this method and the absence of any serious side-effects

In the meantime *Margulies* of Mt. Sinai Hospital in New York, who had been experimenting for some time with plastic materials, devised a polyethylene spiral, now distributed under the trade name Gynekoil. A little later Dr *Lippes* of the University of Buffalo School of Medicine, developed a new polyethylene device in the shape of a double S—known as the Lippes Loop a

obtain information on newly married couples, with particular references to the trends of migration into and out of the area.

In the village area, the contraceptives supplied in the beginning were condoms foam tablets and diaphragm and jelly These were issued at the clinic which was incorporated with the Child Welfare Clinic. The methods were taught at the post-natal check up *i.e* within 6 weeks of delivery Other cases motivated by midwives in the field or social workers were also given their supplies here. As the numbers grew it was thought advisable and convenient to do the distribution of foam tablets and condoms in the field by the midwives on their routine home-visits, the idea being to integrate family planning into the existing well-organised Maternal and Child Health services of the country

In 1964 an evaluation of the work done showed that the birth rate in the village area had dropped from about 30 per thousand in 1958 to 20 4 in 1964 It is reasonable to believe that the declining trend is due to the activities of the Project. The age specific birth rate has also shown a falling trend, particularly for the age group 25–35 years.

Encouraged by these results and the experience gained in the village area, the Swedish Project, in the middle of 1965, suggested to the Government of Ceylon to start Family Planning on a nation-wide scale by integrating it into the Maternal and Child Health services of the country and utilising other health facilities available.

Accordingly a Bilateral Agreement was signed in August 1965 for a period of three years. The aim of the Project s activities are

1 To train medical and other personnel
2. To assist and provide most of the equipment and supplies for the Family Planning clinics
3 To carry out research relating to family planning.

The objective is to reduce the birth rate by a third over a period of 10 years 1966–1976

The country was divided into 4 regions, each with a population of 2–3.5 million The Ministry of Health also appointed an Advisory Committee which meets once a month to advise the

Director of Health Services regarding the working and progress of the National Programme.

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third type invented by *Birnberg* consists of two triangles joined at the tip and resembling a bow. There is a heart-shaped device, designed by the Pathfinder Fund Association and a host of others.

Unlike the Grafenberg silver ring which had to be removed periodically, the new plastic devices are inert and can remain in the uterus indefinitely. They are flexible and can be inserted without dilatation of the cervix, hence no anaesthesia is necessary.

To facilitate removal of the I.U.D. and to determine whether it is in place, Lippes attached an appendage of two nylon threads to the loop and Margulies had plastic beads on his coil—extending through the cervical canal into the vagina.

Seen in the light of the alarming rate of population growth, what is required is a contraceptive device that is cheap, harmless, reliable, non repetitive, not related to time of sex union and reversible.

The I.U.D., though not perfect, is nearest this ideal.

Clinical Observations. The study at Bandaragama includes a group of 2018 women who had their first insertions with Lippes Loop C during the period May 1964 to May 1967.

Propaganda and Motivation. Ceylon is believed to have the best Maternal and Child Health services in South-East Asia. Trained midwives are posted even in the most remote areas and they are in very close touch with the Productive Family Group—as part of their routine duties, they pay ante-natal and post-natal visits and assist at home deliveries; they also assist at the Child Welfare Clinics and are responsible for immunisations, etc. Thereby the midwife becomes, more or less, a friend of the family and she it is that has the opportunity to motivate the mother.

All available contraceptive methods are presented to the mother. When the I.U.D. was the method of choice, the woman was referred to the clinic with a note from the midwife. Some of the women, however, were self referred or sent by their friends, who already had the loop themselves.

The women were advised to attend the clinic soon after or at the tail-end of the menstrual period without sex union as the cervix is partially dilated at that time and there is no risk of

introduction into a pregnant uterus. The device, however was introduced at any stage of the cycle, provided there was no union.

In cases of lactational amenorrhoea, insertions were done six weeks to two months after delivery

A preliminary pelvic examination is done to exclude any contra-indications, *e.g.* suspected pregnancy history of acute or subacute pelvic inflammatory disease, signs of pelvic inflammation at the time of examination, presence of fibroids, cancer of the cervix clinically evident or suspected history of recent menorrhagia or metrorrhagia. Cervicitis was not considered a contra-indication to insertion, if there was no sign of infection.

The wearers are taught to examine themselves with washed hands in a squatting position in order to feel the thread before they leave the clinic and are advised to check once in two or three days, especially after the menstrual period, before sex union.

According to age the distribution of cases are

43 per cent under 30 years of age
50 per cent between 30 and 40.

There were no nulliparae that came under our observation. It will be observed that the percentage of younger women accepting the device is now increasing (Table I)

As regards the evaluation of insertions by living children nearly 80 per cent had given birth to three or more living children with an average of 4.5 live births per mother (Tables II, II a, II b)

Table I. Distribution of Cases According to Age

Age group	No of Cases	Per cent
15–19	9	0.5
20–24	319	15.9
25–20	546	27.3
30–34	625	30.9
35–39	416	20.2
40–44	101	5.1
45 & over	2	0.1
	2018	100.0

Bandaragama *I.U.D. Acceptors: Per cent by Age by Year 1964-67*

Age	May–Dec 1964	Jan–June 1965	July–Dec 1965	Jan–June 1966	July Dec 1966	Jan–May 1967
19	–	0.3	0.7	0.4	0.9	–
20–24	12.1	13.8	14.8	17.5	17.4	22.0
25–29	24.6	23.9	28.4	26.1	27.5	33.3
30–34	31.8	34.4	32.0	32.5	27.8	22.0
35–39	21.6	23.0	19.7	19.5	21.2	20.6
40+	9.8	4.6	4.5	4.1	5.2	2.1
Total	100.0	100.0	100.0	100.0	100.0	100.0

Note Because of rounding, per cent totals will not always be 100.

Table II *Evaluation of Insertion by Living Children*

Living Children	No. of Cases	Per cent
1	126	6.2
2	297	14.7
3	384	19.3
4	406	20.1
5	315	15.6
6	219	10.8
7	135	6.6
8	1	3.5
9+	65	3.2
Total	2018	100.0

Table II a. *Evaluation of Insertion by Parity*

Parity	No. of Cases	Percentage
1	102	5.0
	251	17.4
3	32[illegible]	16.2
4	34	1[illegible]1
5	354	17.5
[illegible]	[illegible]	11.[illegible]
7	17[illegible]	8.7
8	94	4
9+	145	[illegible]
Total	[illegible]018	100.0

Table II b. I.U.D. *Insertions by Age/Parity*

Parity / Age	0	1	2	3	4	5	6	7	8	9	Total
15–19		4	5			–	–	–		–	9
20–24		53	112	92	42	14	4		–	–	319
25–29		24	87	133	120	99	43	31	5	4	546
30–34		14	40	81	134	149	98	58	23	28	625
35–39		5	7	21	37	60	69	71	54	72	416
40–44				1	9	12	13	15	10	41	101
45						–	–		2		2
Total		102	251	328	342	354	227	175	94	145	2018

Nearly two thirds of the women wish to limit their families rather than space (Table III) A summary of trends 1964–1967 shows

a younger women are accepting the I.U.D

b. a greater proportion accepting the I.U.D for limiting of family size.

c family size preferences are decreasing.

The data obtained during recent years of reports published concerning the modern I.U.D shows that the method has its limitations

What are the side-effects or complications?

Expulsions (Table IV) include complete expulsions into or from the vagina or partial expulsions requiring removal of the I U D f om the cervix, whether noticed or unnoticed by the wearer In our observations, out of a total of 303 first expulsions, 200 or nearly two thirds had the device re-inserted. In the case of re-insertions Loop D which is slightly larger than C was used

Of 47 second expulsions, 30 had re-insertions and of 14 third expulsions 9 had re-insertions.

The majority of first expulsions took place in the period 0–5 months, but it will be observed that there were almost an equal number which occurred in the period 6–12 months after insertion

Bandaragama I U.D *Acceptors. Per cent by Age by Year 1964–67*

Age	May–Dec 1964	Jan–June 1965	July–Dec 1965	Jan–June 1966	July–Dec 1966	Jan–May 1967
19	–	0.3	0 7	0 4	0.9	–
20–24	12.1	13.8	14.8	17.5	17 4	22.0
25–29	24.6	23.9	28.4	26.1	27.5	33.3
30–34	31.8	34 4	32.0	32.5	27.8	22.0
35–39	21.6	23 0	19.7	19.5	21.2	20.6
40+	9.8	4.6	4.5	4 1	5.2	2.1
Total	100.0	100.0	100.0	100.0	100.0	100.0

Note Because of rounding, per cent totals will not always be 100

Table II. *Evaluation of Insertion by Living Children*

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6	219	10.8
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8	71	3.5
9+	65	3.2
Total	2018	100 0

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6	227	11.2
7	175	8 7
8	94	4 7
9+	145	7.2
Total	2018	100.0

Removals have been done in 126 cases (Table IV) for the following reasons.

Bleeding. The most important medical reason for removal is bleeding, including spotting over long periods. Most of the side-effects not requiring removal also come under this category as will be seen in Table V. Of a total of 126 removals for various reasons, 33 per cent come under this heading.

Table IV *Removals by Reason and Months of Use*

Reasons	Months of Use											Total
	0–2	3–5	6–8	9–11	12 14	15–17	18–20	21 23	24–26	27 29	30+	
Bleeding	10	9	8	6	2	2	4		2	1	3	47
Abdominal Pain	2		1						–	–		3
Wants Baby			2	2	6	4	5	2	–	4	1	26
Leucorrhoea	1				1	1						3
Husband's Request	3	4		1	1	2		2				13
Personal	1	4		2	2		1				2	12
Medical		1	2				2	1	–	1	1	8
Pregnant		1	4	1	1		1					8
Irregular Periods		3									1	4
Others	1		1							–	–	2
Infection				N		1		L				
Total	18	22	18	12	13	9	13	5	2	6	8	126

Table V *Side Effects not Requiring Removal*

Spotting	125
Rich Periods	111
Bleeding	9
Irregular Periods	36
Leucorrhoea	11
Back Ache	11
Abdominal Pain	12
Others	13
Total	328

Table III. Bandaragama. *I.U.D. Acceptors by Intent to Space or Limit Child Bearing by Year 1964–67*

Year	Space	Limit	Total
1964 (May–Dec)	52 (19.7 %)	211	263
1965	177 (23.5 %)	575	752
1966	254 (29.7 %)	599	853
1967 (Jan–May)	47 (33.3 %)	94	141
	530 (26.3 %)	1479	2009

Information incomplete for 9 acceptors

Year	Limiters Number of Living Children				
	1–2	3–4	5+	Total	
1964 (May–Dec)	4.7	32.7	52.5	100	(N=211)
1965	4.7	42.2	53.0	100	(N=575)
1966	6.3	42.7	50.9	100	(N=599)
1967 (Jan–May)	10.6	48.9	40.4	100	(N=94)

Table III a. *Expulsions by Months of Use and Re-Insertions*

	1st Expulsion	Re-Insertion	2nd Expulsion	Re-Insertion	3rd Expulsion	Re-Insertion
0–2	78	51	18	16	5	4
3–5	68	46	12	4	2	2
6–8	41	30	8	5	2	2
9–11	33	22	3	3	–	–
12–14	43	27	4	1	4	1
15–17	19	10	2	2	–	–
18–20	14	9	–	–	1	
21–23	7	4	–	–	–	–
24+	6	–	–	–	–	–
Total	308	199	47	31	14	9

Investigation showed that most of the expulsions occurred around the time of the menses and were complete rather than partial.

Tietze, in his analyses showed an inverse relationship between parity and age to expulsion rates. The age of the woman, how ever appears to be a stronger factor

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Reasons	Months of Use											Total
	0–2	3–5	6–8	9–11	12 14	15–17	18–20	21–23	24–26	27 29	30+	
Bleeding	10	9	8	6	2	2	4		2	1	3	47
Abdominal Pain	2		1						–		–	3
Wants Baby			2	2	6	4	5	2		4	1	26
Leucorrhoe	1				1	1						3
Husband' Request	3	4		1	1	2		2			–	13
Personal	1	4		2	2		1				2	12
Medical		1	2				2	1		1	1	8
Pregnancy		1	4	1	1		1					8
Irregular Periods		3									1	4
Others	1		1									2
Infection				N		1		L				
Total	18	22	18	12	13	9	13	5	2	6	8	126

Table V *Side Effects not Requiring Removal*

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Rich Periods	111
Bleeding	9
Irregular Periods	36
Leucorrhoea	11
Back Ache	11
Abdominal Pain	12
Others	13
Total	32[illegible]

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Information incomplete for 9 acceptors

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6–8	41	30	8	5	2	2
9–11	33	22	3	3	–	
12–14	43	27	4	1	4	1
15–17	18	10	2	2	–	
18–20	14	9	–	–	1	
21–23	7	4	–	–	–	–
24+	6	–	–	–	–	–
Total	308	199	47	31	14	9

Investigation showed that most of the expulsions occurred around the time of the menses and were complete rather than partial.

Tietze in his analyses showed an inverse relationship between parity and age to expulsion rates The age of the woman however appears to be a stronger factor

sertion. The bleeding usually stops after the second menstrual period after insertion. This was more marked in anaemic wearers. Calcium and Iron Tablets were given and mostly reassurance to tide the patient over the first three months of use. The bleeding is not usually of an alarming quantity but sufficient to create fear and anxiety in the patient.

Irregular periods. 36 had irregular periods for some months following insertion but they did not want the device removed on that score.

The other side-effects were back-ache, leucorrhoea and abdominal pain.

Follow-Up Wearers are advised to return for follow-up one month after insertion, the main reason being to see how they have reacted to the device, i.e. whether they have had any side-effects or not.

The second visit is three months after the first, because most of the side effects, if any have subsided by that time. They are advised to report once in six months or so in order to calculate the woman months of use.

Prior to this present evaluation of our work, since the follow-up was considered incomplete, a special follow-up survey was organised. The statistician of the Project devised a special Form and a house-to-house survey was conducted by trained workers under her supervision.

By this means we were able to get accurate information about the cases. Many of the cases who came from outside our area were contacted by mail and surprisingly enough, many of them replied. It might be mentioned that a large number of this group were midwives and nurses who had got the insertion done at Bandaragama during their training period.

Many of the wearers who did not turn up for their follow-ups were those who had no complaints at all and did not 'bother —a few however were those who had expelled the device and took another method or gave up family planning methods altogether

Sixty two in this whole group, i.e. 6 per cent, were lost to all follow-up

Closed cases (Table VI) These rates are based on all events—expulsions, pregnancies and removals.

Abdominal Pain. Only three women had the device removed for abdominal pain. In passing, I might mention that we had very little experience of abdominal pain or cramps after insertion.

Planning pregnancy 27 were planning pregnancies—mainly after the lapse of a year or more except in a few cases where the age of the last child was over two years.

Husband's objections Mainly in those cases where the wife had the device inserted without the husband's prior knowledge

Medical Reasons for removal other than bleeding include pre-existing and intercurrent conditions requiring treatment clearly unrelated to the I U D as well as vague complaints related to the I.U.D by the patient.

Accidental Pregnancies. In this group are included those accidental pregnancies that occur with the loop in situ or with the device undetermined *i.e* after an unnoticed expulsion. Of the 27 pregnancies that occurred in the group under observation, 20 were with the loop *in situ.*

Of these 8 had the loop removed on request this was done easily 14 had normal deliveries.

2 ended in abortions (these were not the ones that had the loop removed)

1 left the area and was lost to follow-up

1 miscarriage—5th month.

2 had not delivered at the end of 1967

Of the 7 with the device undetermined 6 had normal deliveries.

1 had not delivered at the end of 1967

Ectopic pregnancies. None were observed in this group

Perforations. None were recorded in this series.

Infection There were no cases of pelvic infection observed in this series

Side-Effects 16 per cent of the cases under observations had side-effects, which did not warrant removal (Table V)

Spotting 125 cases or about 6 per cent had spotting of varying duration *i.e* from a few days to a month or even longer

Rich Periods. Bleeding is a bit of a problem 111 had rich periods which occur generally for the first two months after in

In the first year the first expulsion rate is 15.8 (rather high) but closure rate is only 6.2.

Total closure rates are 11.3 at the end of 12 months and 19.8 at the end of 24 months, so that the corresponding continuation rates are 88.7 and 80.2.

Table 7 shows a comparison of net cumulative continuation rates per 100 acceptors in Bandaragama and other various programmes, i.e. in Co-operative Statistical programme for evaluation of Intra-Uterine Devices.

Our continuation rates are good—88.7 at the end of 12 months and 80.2 at the end of 24 months.

SUMMARY

The chief advantage of the I.U.D. as a contraceptive method is that once the insertion is done and as long as the I.U.D. remains in place no further contraceptive action is required.

Other points in its favour are

a. high level of protection
b. easy insertion and removal
c. low cost of production
d. complete stability under all climatic conditions.

The I.U.D. has been endorsed as a method of contraception by national and international health authorities.

The I.U.D. is particularly advantageous to a population that is not accustomed to contraceptive practice, is economically disadvantaged, where literacy rate is low and medical services in short supply.

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Tabel VI. *Net Cumulative Event and Closure Rates by Type of Termination Continuation Rates and Cumulated Months of Use by Duration of Use—Bandaragama Ceylon*

Type of Termination	Rate per 100 Women	
	12th Month	24th Month
Events		
Pregnancy	0.6	1.8
Expulsions		
First	15.8	23 1
Later	2.0	5.4
Removals		
Medical	3 1	4 6
Planning Pregnancy	0.2	1.3
Other Personal	1 4	2.7
Months of Use Cumulated	6810	13,107
Closures		
Pregnancy	0.6	1.6
Expulsions		
First	6 2	9 7
Later	0.5	2.2
Removals		
Medical	2.5	3 0
Planning Pregnancy	0.2	1.3
Other Personal	1.3	2.0
Total Closures	11.3	19.8
Continuation Rate	88.7	80.2

Table VII. *Net Cumulative Continuation Rates per 100 Acceptors at 1 2 and 3 Years in Various Programmes*

	1 year	2 years	3 years
Bandaragama	88.9	80 2	-
CSP (U.S)	78.5	66 6	58.5
Taiwan	68 8	57 7	-
Thailand	78.0	59.6	-
Pakistan	78 6	61.2	-

Co-operative Statistical Programme for the Evaluation of Intra-Uterine Devices

Source Retention of I U D:s An International Comparison Studies in Family Planning, Number 18 April 1967

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Planning Pregnancy	0.2	1.3
Other Personal	1.4	2.7
Months of Use Cumulated	8810	13,107
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Other Personal	1 4	2.7
Months of Use, Cumulated	6810	13 107
Closures		
Pregnancy	0 6	1.6
Expulsions.		
First	6 2	9 7
Later	0.5	2.2
Removals		
Medical	2.5	3.0
Planning Pregnancy	0.2	1.3
Other Personal	1.3	2.0
Total Closures	11.3	19 8
Continuation Rate	88.7	80.2

Table VII *Net Cumulative Continuation Rates per 100 Acceptors at 1 2 and 3 Years in Various Prog ammes*

	1 year	2 years	3 years
Bandaragama	88.9	80 2	–
CSP (U.S)	78.5	66 6	58 5
Taiwan	68.8	57 7	–
Thailand	78 0	59 6	
Pakistan	78 6	61 2	–

Co-operative Statistical Programme for the E aluation of Intra Uterine Devices

Source Retention of I U D.s An I ternational Comparison Studies in Family Planning, Number 18 April 1967

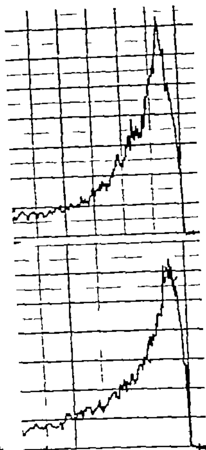


Fig. 1 Bilaterally normal renogram.

33 ovarian tumour in 15 and inflammatory adnexal disease in 13 The interval from hysterectomy to the follow-up examination varied from a fortnight to 18 months. In 80 per cent, a minimum f 4 months had elapsed from the operation. Sixty-five of the ents seen at the follow-up had been operated on by a quali- ynecologist and 215 by a house surgeon, specializing in logy The abdominal hysterectomies by the qualified ts were mostly carried out using the extrafascial reas the others were almost without exception per intrafascial method.

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RENOGRAPHIC STUDIES OF URETERIC PATENCY FOLLOWING TOTAL HYSTERECTOMY

BY

OSMO KOSKELA, JUHA KOKKONEN AND JAAKKO VAHALA

It is known that radical hysterectomy for cancer of the uterine cervix exposes the ureters to a risk of injury Ureteric lesions are sometimes seen in the form of fistulae even after hysterectomy for benign gynaecologic condition they pose the gynaecologist a considerable therapeutic, but sel. om any diagnostic problem. The part played by hysterectomy in .he aetiology of operative ureteric lesions is aptly illustrated by *Higgins* material (1967) with its 87 postoperative ureteric lesions of which 50 followed hysterectomy

Follow-up ([illegible] us after hysterectomy for mainly benign conditions (*Mo,*ʼ*ison* 1960 *Solomons et al.* 1960 *Dahle et al* 1962) have revealed transient and sometimes permanent, ureteric lesions which may have been completely symptom-free or given such mild symptoms as would have been overlooked without a routine follow-up examination of the ureters postoperatively The earlier methods of examination were intravenous pyelography (*Morrison* 1960 *Solomons et al.* 1960 *Dahle et al.* 1962) or cystoscopy with intravenous pyelography only in selected cases (*St Martin et al.* 1953 *Freda and Tacchi* 1962)

Gerble and *Flanagan* (1962) and *Brattenberg et al.* (1965) used radioisotope renography to examine postoperati e ureteric conditions The indicator they used was Hippuran[a] labelled with I-131

The reliability of radioisotope renography for examination of ureteric capacity to convey urine was demonstrated, by com-

paring it with the pyelographic findings, e g by *Denneberg* (1960), *Zum Winkel* and *Scheer* (1960) and *Lawrence et al.* (1963) It is mainly the declining part of the renographic curve which is considered to indicate the ureteric capacity for con veying urine. The curve may decline very slowly suggesting an incomplete obstacle to urinary flow If the curve goes on rising throughout the examination, there is a complete obstruction to urinary flow while demonstrable renal function still exists (*Winter*, 1963 *Zum Winkel* 1964)

Isotope renography is believed to reveal any disorders of ureteric flow even earlier than intravenous pyelography and this method is considered highly suitable for observation of the emptying of the upper urinary tract (*Zum Winkel et al* 1960 *Denneberg*, 1960 *Feine* and *Bauer* 1961 *Gerble* and *Flanagan* 1962 *Braitenberg et al* 1965)

The purpose of the present study was to illustrate with the aid of isotope renography the incidence of latent and permanent ureteric lesions possibly produced by total hysterectomy in treatment of benign conditions

Material

During the period Dec. 1 1965 to Aug 31 1967 total hysterectomy for benign diseases was carried out at the Department of Obstetrics and Gynaecology of Oulu Provincial Hospital on 300 patients either vaginally or by laparotomy All the 300 con secutive patients of whom 222 had been operated on by the abdominal and 78 by the vaginal route were invited to undergo follow-up examination in order to verify the condition of their ureters by isotope renography 280 patients (93 3 per cent) at tended the follow up examination in 76 hysterectomy had been performed by the vaginal route Vaginal hysterectomy had always been accompanied by vaginoplasty Of the patients treated by laparotomy 56 had undergone hysterectomy only 35 hysterectomy and unilateral salpingo-oophorectomy while in 113 cases both adnexae had been removed at the time of hysterectomy

The principal diagnoses at laparotomy were uterine fibroids in 112 cases, recurrent irregular bleeding in 31 endometriosis in

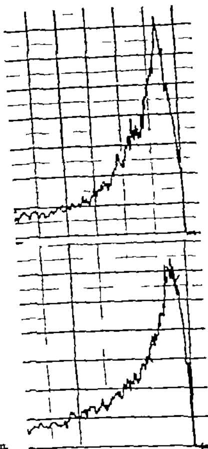


Fig. 1 Bilaterally normal renogram.

33, ovarian tumour in 15, and inflammatory adnexal disease in 13. The interval from hysterectomy to the follow-up examination varied from a fortnight to 18 months. In 80 per cent a minimum of 4 months had elapsed from the operation. Sixty-five of the patients seen at the follow-up had been operated on by a qualified gynecologist and 215 by a house surgeon, specializing in gynaecology. The abdominal hysterectomies by the qualified gynecologists were mostly carried out using the extrafascial method, whereas the others were almost without exception performed by the intrafascial method.

paring it with the pyelographic findings e.g by *Denneberg* (1960) *Zum Winkel* and *Scheer* (1960) and *Lawrence et al* (1963) It is mainly the declining part of the renographic curve which is considered to indicate the ureteric capacity for conveying urine The curve may decline very slowly suggesting an incomplete obstacle to urinary flow If the curve goes on rising throughout the examination, there is a complete obstruction to urinary flow while demonstrable renal function still exists (*Winter* 1963 *Zum Winkel* 1964)

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Material

During the period Dec. 1 1965 to Aug. 31 1967 total hysterectomy for benign diseases was carried out at the Department of Obstetrics and Gynaecology of Oulu Provincial Hospital on 300 patients either vaginally or by laparotomy All the 300 consecutive patients of whom 222 had been operated on by the abdominal and 78 by the vaginal route were invited to undergo follow-up examination in order to verify the condition of their ureters by isotope renography 280 patients (93 3 per cent) attended the follow-up examination In 76 hysterectomy had been performed by the vaginal route Vaginal hysterectomy had always been accompanied by vaginoplasty Of the patients treated by laparotomy 56 had undergone hysterectomy only 35 hysterectomy and unilateral salpingo-oophorectomy while in 113 cases both adnexae had been removed at the time of hysterectomy

The principal diagnoses at laparotomy were uterine fibroids in 112 cases, recurrent irregular bleeding in 31 endometriosis in

determined beforehand by X-ray examination. The collimator tubes were placed against the probable sites of the kidneys, and their position was changed in the early phases of the examination if the site of maximum activity differed from their initially assumed positions. Abnormal renograms were repeated, and if the curve was still pathological on either side the condition of the ureters was verified by intravenous pyelography (Figs. 1 and 2).

Results

The results are presented in Table I. The renograms of 241 patients were normally shaped. For another 26 the repeated renograms were normal. In all these cases the half time of activity bilaterally was less than 18 minutes. The remaining 13 patients underwent intravenous pyelography with special attention focused on the condition of the ureters along their whole course to the bladder. The ureters were normal in the pelvic region of all but one of these 13; in this case the right ureter deviated medially but was patent and smooth-walled. A unilateral mobile kidney was detected in two patients, a large calculus in the renal pelvis in one, while renal changes caused by chronic pyelonephritis were noted in two patients. One patient had ureteric narrowing at the junction between the renal pelvis and the ureter, which explained the repeatedly abnormal unilateral renogram (Fig. 2). Two patients had high blood pressure and one compensated cardiac failure. In five patients whose renograms repeatedly showed a pathological shape, mainly an abnormal ascending part, intravenous pyelography offered no explanation for the changes in the renogram. Group I of Table I included a patient in whom a ureterovaginal fistula caused by total hysterectomy had been

Table I. *The Total Hysterectomy Series Examinated Postoperatively by Renography and Intravenous Pyelography*

First postoperative renogram bilaterally normal	241
Follow-up renogram bilaterally normal	26
Ureteric condition verified by intravenous pyelography and ureters in the pelvic region found normal	13
Total attending follow-up examination	280

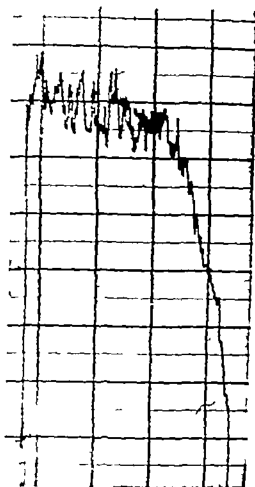


Fig. 2. Renogram of a patient in whom ureteric narrowing was found at the borderline between renal pelvis and ureter

Method

The patients seen for follow up examination were subjected to radioisotope renography using Hippuran® (sodium-ortho-iodo-hippurate) labelled with I 131 0.5 μCi per kg of body weight was injected into the cubital vein The examination was carried out in the supine position, and the variation of activity in both renal regions was observed separately by external measurements for 15–30 minutes. The activity recorder used was Berthold's two-channel renograph. Its scintillation detectors were equipped with sodium iodide crystals activated by thallium. The collimator openings were 5 cm in diameter The site of the kidneys was not

Table II. *Incidence of Ureteric Damage in three Consecutive Series of Total Hysterectomies*

Author	Number of Operations	Permanent Latent Lesions	Transient Lesions	Fistulas
Solomons et al.	191	3	2	
Dahle et al.	98	1	not reliably examined	2 (one vesico-vaginal)
Koskela et al.	280		not reliably examined	1

Abdominal total hysterectomy is considered to constitute a graver risk to the ureters than the vaginal operation, mainly due to the different indications for these two operations. The absence of latent ureteric lesions in the present series may be attributed partly to the fact that in our hospital the less experienced surgeons always carried out abdominal hysterectomy for benign diseases by the intrafascial method.

Isotope renography is a simple, quick and reliable examination involving no discomfort to the patient. Without any preparations, it may be used to verify the condition of the ureters postoperatively. Patients in poor condition, and even patients sensitive to contrast media who therefore cannot undergo intravenous pyelography can be examined by this method. The amount of irradiation involved in isotope renography by Hippuran is considered negligible. Qualitative assessment is considered reliable in the interpretation of the curves (*Gerble* and *Flanagan* 1962, *Winter* 1963, *Koplowitz et al.* 1965). Besides qualitative assessment, the half-times of the renograms were also measured in the present follow-up examination: those not exceeding 18 minutes, according to *Zum Winkel* (1964), were considered normal.

Although no latent, permanent ureteric lesions were detected in the present series the literature seems to justify the statement by *Feda* and *Tacchi*: "Whether or not an incidence of unrecognized non-functioning ureters of almost 2 per cent is suf

repaired by a successful operation prior to the follow-up examination. The fistula had originated in connection with extra-fascial hysterectomy performed by laparotomy

Discussion

In symptom-free operative ureteric lesions, diagnosis can only be reached by routine examination of the postoperative condition of the ureters. Reliable statistics, analysing the effect of hysterectomy on the ureters must be based on material including a preoperative examination. Hysterectomies for malignant growths are not accepted for inclusion in series of this type (*Solomons et al.* 1960 *Dahle et al.* 1962)

Only 6.7 per cent of the patients invited failed to attend the postoperative follow-up examination of the ureters. The present material may therefore reliably be considered to represent the effect on the ureters of 300 consecutive total hysterectomies for benign diseases. The follow-up examination revealed not one latent ureteric lesion. The lack of preoperative examination of the ureters of the present patients is therefore of no importance

It is difficult to find in the literature any comparable statistics on ureteric lesions caused by total hysterectomy although routine postoperative examination has been carried out in many series. The series reported on by *St Martin et al.* (1953) *Morrison* (1960) and *Freda* and *Tacchi* (1962) contained cases of malignancy Both these series and that reported on by *Solomons et al* (1960) also contained other gynaecological operations. The routine examinations on the patients of *St Martin* and of *Freda* and *Tacchi* were carried out by cystocopy a method which can hardly be considered fully reliable unless there is a complete obstruction. Moreover the comparability of the material, is reduced by the different intervals between operations and postoperative examination. The series by *Dahle et al.* and *Solomons et al* were most comparable to the present material (Table II) In the series by *Dahle et al* and in the present material, the late follow-up examination cannot be taken to have traced conclusively any transient ureteric lesions there may have been.

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STRESS INCONTINENCE

A Follow-up Study of Operative Treatment

BY

L. SOLEM SKJÆRAASEN

Introduction

The term stress incontinence is used for the common condition in women, in which there is involuntary loss of urine on straining, coughing, sneezing or laughing. *Kegel* (1948) has claimed good results for his method of pelvic floor exercises, but the general opinion is still that the treatment must be operative. Most gynaecologists consider that the first operation for stress incontinence should be a simple vaginal plastic procedure, mostly a modification of the method described by *Kelly* (1913) For subsequent operations different methods are recommended. Opinions vary as to the failure rate of vaginal repair operations.

At the Department of Obstetrics and Gynaecology University of Oslo a large number of patients with stress incontinence are operated on each year The patients are referred from every part of Norway and among these several have already had operations in their local hospitals. A follow-up study has been carried out with particular attention to the results of different types of operation, the results of subsequent operations and the time taken for the symptom to recur

Material and Methods

In a 10-year period (1955 to 1964) 725 patients with stress incontinence were operated on at the Department of Obstetrics and Gynaecology University of Oslo Questionnaires were sent to these 725 women in 1966 and information was obtained from 627 From this number 72 cases had to be excluded. Forty of the women were dead, two had severe senile dementia, and 30

ficient to justify routine postoperative diagnostic measures will remain for each individual gynaecologic surgeon to determine. Routine follow-up examination was omitted as long as the only reliable routine examination was intravenous pyelography. Not until the introduction of isotope renography has reliable post operative follow up examination been possible in practice.

SUMMARY

An unselected series of 280 patients treated for benign gynaecological conditions by total hysterectomy is presented. The condition of their ureters was examined postoperatively by isotope renography. No instance of a permanent, latent ureteric obstruction was found in the series.

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Three-hundred-and-fifty of the women had suffered from stress incontinence exclusively while 93 had combined stress and urge incontinence and 112 had stress incontinence with other urinary symptoms such as frequency nocturia, dysuria or incontinence in a recumbent position. Patients with urge incontinence alone were treated conservatively

Ninety-eight cases out of the 555 women in the follow-up study had a history of one or more previous vaginal operations for stress incontinence. Seventy-nine had been operated on once eighteen twice and one three times before. Twenty of our own patients had subsequent operations in our department in the 10 year period and in these cases only the last operation has been considered.

The preoperative findings on pelvic examination are shown in Table II. Cystocele was the most common finding in the patients

Table II. The Findings at the Preoperative Pelvic Examination before I First Operation II Second Operation III Third Operation

Findings		No of cases
Cystocele	I	261
	II	17
	III	3
Prolapse of the uterus	I	96
	II	12
	III	2
Descent of the urethra	I	64
	II	13
	III	1
Vaginal scarring	II	37
	III	12
		1
Other pathological condition (see text)	I	10
Normal findings on pelvic examination	I	26
	I	457
	II	79
	III	18
Total		555

One woman had been operated upon three times befor

patients who according to their records had operations for both stress incontinence and genital prolapse denied any trouble with stress incontinence preoperatively Thus the follow-up study includes 555 patients

The diagnosis of stress incontinence in this series was based on the history Cystoscopy and cystometry were performed routinely Lateral urethrocystograms were obtained in 24 patients only and showed loss of the posterior urethro-vesical angle in all but one

Table I shows the age distribution in the follow-up study Patients between 40 and 59 years constituted the largest group. Only four per cent were older than 70 and 13 per cent less than 40 years. The youngest patient was 23 and the oldest 81 years of age.

It is generally accepted that the stress incontinence is related to pregnancy and damage to the genital tract at the time of delivery Congenital forms are rare Among the 555 women in this study only 9 were nulliparous. The largest group – 192 patients – had given birth to two children. Many of them may have developed stress incontinence at the time of their first delivery It is reasonable to assume that the records do not give complete information about obstetrical complications Out of the 555 cases 88 had been delivered by forceps, three cases had a complicated delivery with total rupture of the perineal body and four had been delivered by Caesarean section Thirty patients had children weighing more than 4500 grams at birth. One hundred and fifty five women stated that they developed stress incontinence immediately after a delivery

Table I The Age Distribution of the 555 Patients in the follow-up Study

Age in Years	No of Cases
<30	10 (1.8 %)
30–39	64 (11.7 %)
40–49	181 (32.6 %)
50–59	179 (32.2 %)
60–69	100 (18.0 %)
>70	21 (3.7 %)
Total	555

in 45 the incontinence was unchanged and five reported aggravation of their symptom after the operation.

Table III. Results of Different Types of Operation for Stress Incontinence (I. First Operation, II. Second Operation, III. Third Operation)

Operation	No. of Cases			
		Total	Continent	Improved
Modified Kelly	I	70	17	28
			64.3 %	
	II	31	8	5
	III	2	1	
Modified Kelly colpoperineorrhaphy	I	139	51	46
			69.8 %	
	II	9	4	2
	III	1	1	
Modified Kelly amp. of the cervix, colpoperineorrhaphy	I	178	87	58
			80.3 %	
	II	11	6	5
	III	2	2	
Modified Kelly amp. of the cervix	I	5	3	
	II	1		1
	III	1	1	
The interposition operation	I	54	29	11
			74.1 %	
	II	18	5	5
	III	7	4	
Marshall-Marchetti-Krantz	I	2	2	
	II	3	1	1
	III	4	2	1
Other	I	9	3	4
	II	6	4	1
	III	1	1	
		1		
		555	232	166

One woman had her fourth operation for stress incontinence with no improvement

amp. amputation

having their first operation Prolapse of the uterus was less frequently combined with stress incontinence Descent of the urethra during straining was found in 64 cases. The group named "other pathological conditions comprises patients with retroversion of the uterus small fibromyomas and rectocele In 26 patients there was no obvious abnormality on pelvic examination. Vaginal scarring was more common than recurrence of cystocele and prolapse of the uterus in the patients with a history of previous operations.

Nearly all patients had vaginal operations. During the 10-year period a modified Kelly operation was usually preferred. The suburethral dissection was often combined with urethrolysis. With reefing mattress sutures in the urethro-vesical fascia the surgeons have tried to support the bladder neck. Different materials were used for these "Kelly sutures" Non-absorbable material (silk or nylon) was used in 159 patients and in the others catgut was used A survey of the different types of operation is given in Table III.

The same type of operation was usually employed for subsequent operations. The Marshall-Marchetti-Krantz operation was performed in only nine cases, and the group other operations" includes two Aldridge slings and two Ingelman Sundberg operations.

Results

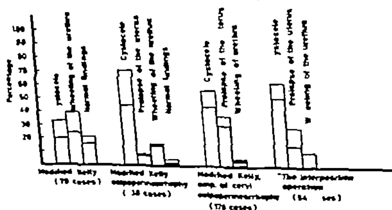
Table IV shows the results of primary and secondary operations postoperatively and at the time of the follow-up study which was done in 1966

The expression postoperatively refers to the time when the patients started a normal life at home According to the records more than 95 per cent of the patients were continent when they left the hospital but this information is of little value It is our experience that patients suffering from stress incontinence often have no leakage of the urine when they live quietly in hospital. Two-hundred-and ninety-nine women were completely continent postoperatively and 87 were improved in the group of primary operations The success rate is therefore 84 3 per cent Twenty one patients gave no information about the postoperative result,

Table V *The Results of the Operations Compared with the Findings on the Preoperative Pelvic Examination in I Primary Operations II. Subsequent Operations*

Findings		No. of Cases Total	Continent	Improved
Cystocele	I	261	116	69
			70.9 %	
	II	20	8	7
Prolapse of the uterus	I	96	44	30
			78 %	
	II	14	9	3
Descent of the urethra	I	64	25	27
			81.2 %	
	II	14	5	1
Vaginal scarring	II	50	18	10
			56 %	
Other pathological findings (see text)	I	10	1	6
Normal findings	I	26	6	13
			73.1 %	
	I	457	192	145
	II	98	40	21

Table VI *The Late Results of the Most Common Types of Primary Operation in the Series Related to the Findings on the Preoperative Pelvic Examination The Grey Part Represents Continent and Improved Patients*



The follow-up study revealed in this group continence in 192 patients and improvement in 145. That means a success rate of 73.7 per cent. In 99 cases the stress incontinence was unchanged and in 10 cases aggravated after the operation. Eleven patients had second operations before this follow-up study was performed.

In Table IV are also seen the results of the subsequent operations. Postoperatively 76.5 per cent were continent or improved compared with 62.2 per cent at the follow up study.

The final results of the different types of operation are shown in Table III and Table V shows the late results related to the preoperative findings on pelvic examination. In Table VI the results are shown in relation both to the type of operation and the preoperative findings.

The success rate of operations among the 350 patients who had stress incontinence only was 74.9 per cent. Among the remaining 205 cases with stress incontinence combined with urgency or other urinary symptoms 66.5 per cent were continent or improved after surgical treatment.

Table IV. *The Condition Postoperatively and at the Follow-up Study in I. 457 Cases of Primary Operation and II. 98 Cases of Subsequent Operations*

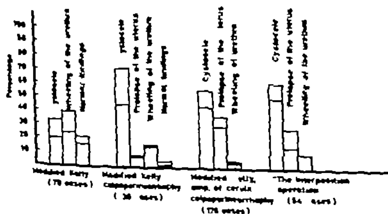
	Postoperatively				
	Continent	Improved	Unchanged	Aggravated	Unknown
Group I	299	87	45	5	21
	84.3 %		15.5 %		
Group II	60	15	14	4	5
	76.5 %		23.3 %		

	At the Follow-up Study				
	Continent	Improved	Unchanged	Aggravated	Further Operation
Group I	192	145	99	10	11
	73.7 %		26.3 %		
Group II	40	21	24	10	3
	62.2 %		37.8 %		

Table V *The Results of the Operations Compared with the Findings on the Preoperative Pelvic Examination in I Primary Operations II Subsequent Operations*

Findings		No. of Cases		
		Total	Continent	Improved
Cystocele	I	281	116	69
			70.9 %	
	II	20	8	7
Prolapse of the uterus	I	96	44	30
			78 %	
	II	14	9	3
Descent of the urethra	I	64	25	27
			81.2 %	
	II	14	5	1
Vaginal scarring	II	50	18	10
			56 %	
Other pathological findings (see text)	I	10	1	6
Normal findings	I	26	6	13
			73.1 %	
	I	457	192	145
	II	98	40	21

Table VI *The Late Results of the Most Common Types of Primary Operation in the Series Related to the Findings on the Preoperative Pelvic Examination The Grey Part Represents Continent and Improved Patients*



The success rate among the patients where non absorbable material was used for the Kelly stitches was 65 4 per cent compared with 75 2 per cent in the remaining group

At the time of the follow-up study 163 patients had recurrent stress incontinence although 79 of these still reported some improvement as a result of their operation. Of the other 84 patients with recurrence 48 reported that they had been completely continent for a while and 36 had shown temporary improvement.

Table VII shows the time interval before the recurrences occurred. The questionnaires from 25 patients gave no information about when their symptoms recurred, and 11 stated that stress incontinence re-appeared gradually after the operation. Of the 79 patients who were considered continent postoperatively and

Table VII. *A Survey of Patients with Recurrence of Stress Incontinence and the Time Interval before Recurrence Occurred*

Condition Post operatively	Condition at the Follow-up Study Compared with the Pre-operative	No of Cases				Average Time before recurrence for the Patients in Group III (Years)
		Total	Group I	Group II	Group III	
Completely continent	Still improved	79	15	6	58	3.3
Completely continent	Unchanged aggravated or had further operation	48	1	2	45	2.2
Improved	Unchanged aggravated or had further operation	36	9	3	24	1.6
		163	25	11	127	

Group I. patients with unknown length of time before recurrence
Group II patients with gradual appearance of recurrence
Group III patients with a known length of time before recurren

who were still improved at the follow-up study 17 women were continent for more than five years, four got leakage of the urine during the first six months after the operation, and the remainder 37 patients – were continent from one to five years postoperatively The average time before the symptom recurred was 3.3 years in this group.

The next group comprises patients with complete continence postoperatively but whose symptom had since completely recurred. The average time before recurrence was shorter in this group – 2.2 years. Eight of the patients were continent for more than five years after the operation, eight got stress incontinence during the first half year after the operation and the remaining 29 had recurrent symptoms from one to five years after the operation. The patients reporting an improvement but not complete cure have the shortest average time before recurrence, 1 6 years. Only one of them was still improved after five years. Nine had a recurrence during the first six months and 14 from one to five years after the operation.

The age distribution among the women with recurrence of stress incontinence seems to be almost the same as in the whole group of patients. There appears to be no higher frequency of recurrences in the menopausal age group Fourteen patients have had a further operation since the follow up and six became continent or were improved.

There were no postoperative deaths and few serious complications (Table VIII) Ninety-one patients had retention of urine for more than one week after the operation. The cure rate among those with temporary retention was only very slightly better than in the group as a whole In most cases the indwelling catheter was removed on the fourth day postoperatively Poor healing of the anterior colporrhaphy was reported in 91 patients. Five patients developed thrombophlebitis and one of these cases was complicated by pulmonary embolism. Urinary fistulae occurred in two patients and both were cured surgically One woman had a strangulation of the ileum after a Marshall-Marchetti-Krantz operation combined with laparotomy

Table VIII. *A Survey of Patients with Postoperative Complications and the Results at the Follow-up Study in These Cases*

Complication	No. of cases		
	Total	Continent	Improved
Retention of urine	91	44	25
		75.8 %	
Poor healing of the anterior colporrhaphy	91	31	26
		62.6 %	
Haemorrhage	21	9	6
Urinary infection	26	10	10
Urethro-vaginal fistula	2	2	
Intestinal strangulation	1	1	
Thrombophlebitis	5	2	1
Other	17	11	6
	254	110	74
		72.4 %	

Discussion

It is difficult to draw firm conclusions from this follow-up study Only 555 out of the 725 patients who had operations for stress incontinence are included in the study Like most series the material is not homogeneous. At the preoperative pelvic examination different conditions were found and the patients had different types of operation and the members of the staff did not always use the same technique The diagnosis of stress incontinence was based mainly on the patient's history and the results had to be evaluated on the subjective impression of the patients. This involves a degree of uncertainty particularly in women stating a postoperative improvement The conclusions therefore have to be guarded

The cure rate which usually refers to continent and improved patients varies in the published reports *Millin* (1948) and *Read* (1950) reported late results a little above 70 per cent cured. In a follow-up study *Nilsen* (1958) found a cure rate of 74 per cent among 110 patients operated on during the years 1950–1954

Björo and *Mejlænder* (1958) found 91.3 per cent of 127 women continent or improved after operation for stress incontinence when a modified Kelly operation with non-absorbable suture material was used. *Jeffcoate* (1961) reported a 60 per cent cure rate for anterior colporrhaphy on the basis of a two years follow-up study. In *Te Linde's* (1962) hands 90 per cent were continent and five per cent improved postoperatively but he did not present the late results. Many gynaecologists claim to have successful results immediately postoperatively in about 90 per cent, however they are aware of the reduced number of cured patients with the elapse of time.

The postoperative results in the present series are poorer than in the above reports, but the longterm results do not differ much. The cure rate in second or subsequent operations is lower than in the primary operations. A number of patients, however have been cured by repeat-operations, even by a third operation. Probably there is a greater risk of stress incontinence being aggravated by a secondary operation. A few of the women who had repeat-operations reported an almost continuous leakage of urine afterwards.

Considering the results in relation to the different types of operation it is obvious both in primary and in secondary operations that the Kelly method has failed in many cases. Where the Kelly operation was combined with a colpoperineorrhaphy and particularly also with an amputation of the cervix, the results were better. This agrees with *Bailey* (1954) who was "holding a firm belief that practically every case of stress incontinence could be cured by an adequately performed Manchester colporrhaphy". The results of the utero-vesico-vaginal interposition operation are rather good. Our experience with other methods is minimal. The outcome by the Marshall-Marchetti-Krantz operation is remarkable. Five out of seven women having repeat-operations of this type were continent or improved at the time of the follow-up study. In our series of cases the Aldridge sling operation was used once for a third and once for a fourth operation, resulting in complete continence and no improvement, respectively. Ingelman-Sundberg's operation was carried out for two repeat-operations with improvement in one of them. The use of

non-absorbable material for the Kelly sutures" can not be shown to have improved the final results in this series.

In the primary operations a higher cure rate was found in patients with descent of urethra and prolapse of the uterus. The numbers of women in the different groups coming to repeat operations are small. Those with vaginal scarring, though a small number showed a tendency to inferior results.

The choice of operation is certainly connected with the preoperative findings on pelvic examination. The figures in Table VI do not show any clear relation between the final results, the type of operation and the preoperative pelvic findings. There are failures in nearly every group Patients with prolapse of the uterus have mainly required treatment by the most favourable operations and that may to some extent explain the higher cure rate in this group

The results seem to be most favourable in the patients who had pure stress incontinence without other urinary symptoms. In the other groups there are probably some patients included who were not suffering from true stress incontinence Operative treatment may have been unwisely chosen in these cases.

The increased recurrence rate as time passes is confirmed. Of the patients with complete continence postoperatively only a few had recurrent symptoms during the first six months, but the deterioration in the results even after five years is remarkable Patients who were only improved by the operation tend to have an earlier return to the preoperative degree of stress incontinence

It has been suggested that recurrences may be due to inadequately performed operations (*Read* 1950) Our study supports this view as a few particularly skilled surgeons achieved better results than those in training who in our series had the poorest results, though as a rule they were assisted by senior gynaecologists.

There were few serious complications It has been assumed that a successful operation causes retention of urine for one or two weeks after the operation The results in the 91 women who had retention of urine for more than one week after the operation are not convincingly superior to those for all patients It has

to be mentioned that some questionnaires indicate continuous micturition trouble with urinary infections. Probably some of these patients still have a reduced ability to empty the bladder completely. In our experience poor healing of the anterior colporrhaphy can lower the cure rate.

The results of this follow-up study show the inadequacy of our surgical treatment of stress incontinence, as one fourth of the patients have no lasting improvement from the operation. Better results might possibly be obtained by more careful selection of patients with more refined diagnostic techniques including routine X-ray examination of the posterior urethro-vesical angle and simultaneous recording of the intra-urethral and intra-vesical pressures (*Enhörning*, 1961). In this series the therapy has been selected only by considering the patients history and the findings on the preoperative pelvic examination. *Green* (1962) claimed to have improved his results by separating the patients in two groups: those with loss of the posterior urethro-vesical angle in one group and in the other group those with rotational descent of the urethra in addition to loss of the angle. In the latter group he had performed a more extensive operation. Patients with great shortening of the urethra may be selected for an anterior urethropexy (*Lapides* 1960).

Nevertheless, the fact that some skilled operators have very good final results, should encourage every gynaecologist to improve his surgical technique.

SUMMARY

A follow-up study of the results of surgical treatment has been undertaken among 555 patients who had operations for stress incontinence at the Department of Obstetrics and Gynaecology University of Oslo, during the years 1955–1964. Nearly all had vaginal operations. A modified Kelly operation was usually preferred, often combined with an amputation of the cervix and a colpoperineorrhaphy. The operation was the primary treatment in 457 cases but 98 cases had a second or subsequent operation. Postoperatively 84.3 per cent were continent or improved after the primary operations compared with 76.5 per cent following

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CANCER OF THE FEMALE URETHRA

A Clinical Study of 25 Cases

BY

EGIL SKJÆRAASEN

Malignant tumours of the female urethra are uncommon. *Zinser* (1955) found that the rate of urethral cancer related to the incidence of cancer in the female genital organs was 0.83 per cent. During the years 1953–65 altogether 28 new cases of urethral cancer in women were reported to the Cancer Registry of Norway. In the same period 4449 cases of invasive cancer of the cervix were detected in the country. The incidence of malignant urethral tumours thus constituted only 0.63 per cent of that of cervical malignancies. This explains why the reported series of patients with urethral cancer have been so small. The largest series published up to 1966 consisted of 79 cases (*Grabstald et al.* 1966).

A variety of treatments have been tried in cancer of the urethra in females. Radium irradiation, external irradiation, or combined radiotherapy and surgery have resulted in 5-year sur-al rates in the range of 30–43 per cent (*Fricke et al.* 1949, *Ruch et al.* 1952, *Monaco et al.* 1958). *Sallinen et al.* (1965) reported on 29 patients treated mainly by electrocoagulation and local radium, and with a 5-year survival of 38 per cent. Treatment principally with local radium has been advocated by several workers, resulting in 5-year survival rates of from 28 to 67 per cent (*Göbel et al.* 1947, *Zinser* 1955, *Staubitz et al.* 1955, *Hultberg* 1956, *Wasserburger* 1956).

Against the background of the small series of cases and the relatively poor results of treatment hitherto reported, it was of

repeat-operations. At later follow-up the cure rate was 73 7 and 62.2 per cent respectively

Many patients with recurrent symptoms had been completely continent for several years, some for more than five years.

There were no postoperative deaths and few serious complications.

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Table II.

Symptoms	No. of Cases
Bleeding	16
Dysuria or frequency	14
Urinary retention	7
Urinary incontinence	2
Tumour in the urethral orifice	2
Discharge	3
Dyspareunia	3

was recorded in 22 cases. One patient had noted spotting of blood for many years. In the other 21 patients symptoms had been present from one month to two years, and on average 8.7 months.

A clinical diagnosis of urethral caruncle had been made in three patients 2–5 years before a biopsy from the lesion disclosed cancer

Cytological smears had been taken from the urethral orifice in only two patients In both cases malignant cells were found.

The tumour was clearly limited to the anterior part of urethra in eight cases (Table III) Vulvo-urethral forms with extension to the adjoining parts of vulva or the outer part of the anterior vaginal wall were seen in four cases. Tumour involving the posterior part of urethra or with extension to the surroundings was found in 13 cases.

Table III *Follow-up Results Related to Location of Tumours*

Location	Total No. of Cases	Alive without Residual Disease	Dead from Intercurrent Disease	Dead from Cancer
Anterior part of urethra	8	2	2	4
Vulvo-urethral	4	1	1	2
Posterior part of urethra	13	2		11
Total	25	5	3	17

interest to review our own series of urethral cancer in the female dating back to the opening of the hospital in 1932.

Material

Between the years 1932 and 1964 29 women with tumours classified as cancer of the urethra were admitted to the Norwegian Radium Hospital. In four cases the histological examination did not confirm the clinical diagnosis and these were therefore excluded. Accordingly the series comprises 25 cases of cancer of the urethra confirmed microscopically.

The age distribution of these cases is shown in Table I. The youngest patient was 42 years and the oldest 73 years of age at the time the diagnosis was established. The majority of the women (19 patients) were in the age group 50–69 years.

Table I. *Age Distribution in 25 Women with Cancer of Urethra*

Age Groups	No of Cases
40–49 years	2
50–59 years	11
60–69 years	8
70–79 years	4
Total	25

Average age: 60.7 years

There were five nulliparous patients three patients had given birth to one child, and six to two children, while seven patients had had three or more deliveries. In four cases the parity was not stated in the records.

Clinical findings

The symptoms are given in Table II Haematuria or spotting of blood with or without painful or frequent micturition were the most common complaints being observed in 24 of the 25 patients. In one case dyspareunia due to narrowing of the vaginal introitus was the only symptom The duration of the symptoms

2400 r to 5000 r During the period 1952–60 orthovoltage X-ray treatment was given to eight patients. The skin doses in these cases were from 3000 r to 3600 r Since 1961 a 31 MeV betatron machine has been used in seven patients, with a calculated tu mour dose of between 4000 r and 6000 r

Follow-up

The follow-up in 1967 of the 25 patients showed that five patients were alive and without recurrence after an observation time from 3 to 6 years. Two patients died of intercurrent disease after 10 years and 17 years of observation respectively The recurrence free group thus consisted of seven cases, one of which was treated by resection of the urethra, one was treated by radium and external betatron irradiation, while the remaining five were subjected to a combination of surgery and radiotherapy Four of the seven recurrence free patients were found in the group of tumours located in the anterior part of urethra (Table III) Only one of the five patients with lymph node metastases was found to be without recurrence

One patient died from pulmonary embolism two weeks after excision of the urethra. Seventeen patients are considered to have died of urethral cancer The average time of survival after the initial treatment was 23 months.

Discussion

The reported series from the Norwegian Radium Hospital confirms that urethral cancer in women is an uncommon disease with a gra e prognosis.

The poor over-all results reported in the literature are the more impressive as observations indicate that urethral cancers are apt to remain localized for a considerable period of time (G *abwald et al* 1966) Crucial points might be that the disease is uncommon and that the symptoms are not alarming and may often be neglected. The small published series indicate that even specialists in gynaecological cancer generally obtain only a limited personal experience of female urethral cancer The varied

Pathology

The majority of the lesions were classified as squamous cell carcinomas (15 cases) Transitional cell carcinoma was found in five cases adenocarcinoma in three cases and undifferentiated malignant tumour in three cases. One of the patients with a squamous cell lesion had been treated four years previously for cancer of the uterine cervix stage I The examination revealed a papillomatous squamous cell carcinoma, and a metastasis from the cervical cancer could not be excluded. Since no other signs of recurrence were found however the case was included in the urethral cancer series

Enlarged inguinal lymph nodes were noted in 13 patients. Groin dissection with histological examination of the nodes was performed in six of these and in five cases tumour metastases were found

Therapy

Treatment consisted of surgery radiotherapy or a combination of both. One cachectic patient with a very advanced cancer had no treatment. Two patients were treated exclusively by surgery eight by radiation alone and fourteen patients were subjected to combined treatment.

Partial resection of urethra was performed in four patients, in two of which diathermy was used. Total excision of the urethra was carried out in seven patients. In five of these some type of urinary diversion was established and plastic reconstruction of urethra was performed in the other two cases.

Radiotherapy consisted of local treatment with radium or external irradiation, separately or in combination. Different types of radium source have been applied both in the urethra directly into the tumour (radium needles) or in the vagina It is impossible retrospectively to calculate tumour doses the amount of radium varying between 750 and 3600 mg hours.

The methods of external irradiation varied considerably in the period under discussion. Teleradium therapy was used in seven patients during the years 1932–1958 The beam was directed to the vulva and the inguinal areas skin doses ranging from

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ical removal of the urethra and external 31 MeV betatron radiation.

It is noteworthy that four of the eight patients with tumours confined to the urethral orifice showed no evidence of recurrence, while only two of 13 patients with tumours in the posterior part of urethra were free from recurrence. *Wasserburger* (1956) found one 5-year survival among six patients with tumours in the posterior urethra whereas 22 of 24 patients with growths in the anterior part of urethra were alive after 5 years. These findings show that the location of the tumour has a great bearing upon the prognosis.

During the last decades local radium treatment in urethral cancer in women has become popular It is likely that the mode of radium application is of the utmost importance. Most authors seem to prefer radium needles (*Göbel et al.* 1947 *Ruch et al.* 1952 *Staubitz et al* 1955 *Wasserburger* 1956 *Monaco et al* 1958) *Grabstald et al.* (1966) have also used interstitial radium treatment according to the technique described by *Henschke et al* (1963) On the other hand extensive surgery including urethrocystectomy or anterior exenteration, has been undertaken in posterior urethral tumours, and the results have been encouraging (*Staubitz et al.* 1955 *Grabstald et al.* 1966)

Metastases to the inguinal lymph nodes were observed in five out of 25 patients. This corresponds well with other authors reporting metastases to the groins in 20–25 per cent of cases (*Göbel et al* 1947 *Wasserburger* 1956 *Sallinen et al.* 1965) *Grabstald et al.* (1966) examined the pelvic lymph nodes in 26 of 79 patients and metastases were revealed in 13 Inguinal metastases have been treated by X-ray irradiation (*Fricke et al.* 1949 *Sallinen et al* 1965) groin dissection (*Monaco et al.* 1958) or irradiation and surgery (*Hultberg*, 1956)

In order to obtain a more reliable basis for the handling of urethral cancer it seems necessary to have larger series with more standardized treatment. The treatment should be centralized in special institutions for cancer therapy Cooperation between such centres might contribute to a better understanding of this form of cancer

and often unsystematic therapeutic methods used in these cases seem to support such a statement.

It is a common finding that patients with urethral cancer have micturition troubles (*Monaco et al.* 1958 *Marshall et al.* 1960 *Grabstald et al* 1966) In the present series all patients except one complained of dysuria frequency or bleeding. It follows that urethral cancer should be considered in adult women with urinary symptoms.

Most tumours are located in the outer part of the urethra (*Fricke et al.* 1949 *Wasserburger* 1956 *Monaco et al.* 1958 *Sallinen et al* 1965) They are easily seen and palpated, and are readily accessible for biopsy The most common type of tumour is a squamous cell carcinoma (*McCrea* 1952) and this suggests that exfoliative cytology can be of value in the diagnosis of urethral cancer In two patients in the present series a cytological smear was taken and malignant cells were found in both. Colposcopy is another method that may be of importance for the early diagnosis of this disease. *Koller* (1966) reported on 42 patients with vulval lesions including three cases of urethral carcinoma and stated that the colpophotographic findings were much of the same type as those found in carcinoma of the cervix.

There has been discussion whether caruncle of the urethra may be related to urethral cancer Carcinomatous changes inside caruncles have been demonstrated (*Ratner et al* 1948 *Marshall et al* 1960) Hence the possibility of malignant development in these lesions can hardly be excluded Malignant tumours on the other hand can be erroneously diagnosed as caruncles. *Marshall et al* (1960) found six cases of carcinoma and three cases of Bowen s disease in 376 cases clinically interpreted as caruncles Accordingly it has been claimed that all urethral growths should be subjected to histological examination (*Menville* 1935 *Walther* 1943 *Marshall et al* 1960)

Treatment in the present series of 25 cases was most often surgery combined with radiotherapy The limited number of cases and the large number of different combinations of treatment preclude definite statements as to the effectiveness of the various therapeutic measures Improved preliminary results, however have been observed since 1964 in a few patients treated by rad

ical removal of the urethra and external 31 MeV betatron radiation.

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SUMMARY

Between 1932 and 1964 25 women with urethral cancer were seen in the Norwegian Radium Hospital. The majority of the patients were treated by a combination of radiotherapy and surgery.

Five patients were free from recurrence after an observation period of 3 to 6 years, and two other patients died of intercurrent disease after several years of observation. Seventeen patients died of spread of the urethral cancer and one of postoperative pulmonary embolism.

The series shows that urethral cancer should be borne in mind in patients with micturition symptoms. Cytological smears are likely to be useful as a screening test in suspicious cases, and colposcopy might be of diagnostic value.

The results of treatment are more clearly related to the site of the tumour than to the actual therapy employed. In growths limited to the anterior urethra well planned irradiation as well as surgery may cure the lesions whereas the poor outcome in tumours located in the posterior urethra indicates that more intensive irradiation or extensive surgical intervention is necessary.

A centralized treatment of patients with urethral tumours in special institutions for cancer therapy is recommended.

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SUMMARY

Between 1932 and 1964 25 women with urethral cancer were seen in the Norwegian Radium Hospital. The majority of the patients were treated by a combination of radiotherapy and surgery

Five patients were free from recurrence after an observation period of 3 to 6 years and two other patients died of intercurrent disease after several years of observation. Seventeen patients died of spread of the urethral cancer and one of post operative pulmonary embolism.

The series shows that urethral cancer should be borne in mind in patients with micturition symptoms. Cytological smears are likely to be useful as a screening test in suspicious cases and colposcopy might be of diagnostic value.

The results of treatment are more clearly related to the site of the tumour than to the actual therapy employed. In growths limited to the anterior urethra well planned irradiation as well as surgery may cure the lesions whereas the poor outcome in tumours located in the posterior urethra indicates that more intensive irradiation or extensive surgical intervention is necessary

A centralized treatment of patients with urethral tumours in special institutions for cancer therapy is recommended.

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